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Full-length Article

Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice

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ABSTRACT

The microbiota-gut-brain axis (MGBA) regulates the reciprocal interaction between chronic inflammatory bowel and psychiatric disorders. This interaction involves multiple pathways that are highly debated. We examined the behavioural, biochemical and electrophysiological alterations, as well as gut microbiota composition in a model of antibiotic-induced experimental dysbiosis. Inflammation of the small intestine was also assessed. Mice were exposed to a mixture of antimicrobials for 2 weeks. Afterwards, they received *Lactobacillus casei DG* (LCDG) or a vehicle for up to 7 days via oral gavage.

Perturbation of microbiota was accompanied by a general inflammatory state and alteration of some endocannabinoidome members in the gut. Behavioural changes, including increased immobility in the tail suspension test and reduced social recognition were observed, and were associated with altered BDNF/TrkB signalling, TRPV1 phosphorylation and neuronal firing in the hippocampus. Moreover, morphological rearrangements of non-neuronal cells in brain areas controlling emotional behaviour were detected. Subsequent probiotic administration, compared with vehicle, counteracted most of these gut inflammatory, behavioural, biochemical and functional alterations. Interestingly, levels of *Lachnospiraceae* were found to significantly correlate with the behavioural changes observed in dysbiotic mice. Our findings clarify some of the biomolecular and functional modifications leading to the development of affective disorders associated with gut microbiota alterations.

1. Introduction

The intestinal microbiota plays an important role in the bidirectional interactions between the central and enteric nervous systems (CNS and ENS, respectively). Thus, the MGBA is involved in the pathophysiological mechanisms underlying chronic inflammatory bowel disorders leading to psychiatric disorders (Collins and Bercik, 2009; Grenham et al., 2011). Indeed, apart from gastrointestinal functions, the microbiota and its metabolites have been suggested to be involved in the modulation of brain functions, such as emotional behaviours (Foster and McVey Neufeld, 2013) stress-related responsiveness (Bravo et al., 2012), pain (Bercik et al., 2012), and food intake (Alcock et al., 2014). Consequently, alterations of the "healthy" microbiota, referred to as dysbiosis, might drive functional and behavioural changes in animals and humans (Bercik et al., 2011; Fond et al., 2015). Therapeutic agents able to

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recover intestinal bacteria have been proposed as a novel strategy to address psychiatric disorders associated with infection or inflammation, besides their inherent application to inflammatory bowel diseases. Probiotics, selected commensal bacteria ingested through diet or in the form of supplements, can lead to health benefits beyond basic nutrition (Dai et al., 2013). Indeed, preclinical studies have shown that probiotics have the potential to reduce stress and stress-related disorders such as depression or anxiety (Bercik et al., 2010; Slyepchenko et al., 2014). Moreover, beneficial effects of different probiotics have been reported in hospitalised subjects suffering from psychiatric diseases as well as in healthy volunteers (Messaoudi et al., 2011a,b).

Interestingly, the recent characterisation of the human microbiome provided new grounds to investigate potential epigenetic mechanisms in neurodevelopmental disorders associated with gastrointestinal pathologies, including autism spectrum disorders (ASDs) or attention-deficit hyperactivity disorder (ADHD) (Fond et al., 2015). Previous reports in children revealed a link between alterations in the gut microbiota and the risk of developing neuropsychiatric disorders in adulthood, pointing to early probiotic treatment as a potentially successful intervention (Frye et al., 2015; Partty et al., 2015; Petra et al., 2015). Exploring the molecular mechanisms at the basis of gut microbiota-brain interactions should lead to improve the therapeutic strategies aimed at managing neuropsychiatric disorders, especially those associated with bowel inflammation. However, the cellular, neurophysiological and biochemical bases through which such improvements can be exerted have been so far poorly investigated.

The endocannabinoid system has been shown to be implicated in various pathophysiological states at both peripheral and CNS level, by exerting pleiotropic effects (Rossi et al., 2014; Cristino et al., 2016; Henry et al., 2017; Mela et al., 2016; Russo et al., 2017). In particular, the dysregulation of endocannbinoid tone and the consequent influence on hippocampal neurogenesis have been suggested to play a role in the aetiology of depression (Boorman et al., 2016). Furthermore, substances elevating endocannabinoid levels have been found to ameliorate signs of IBDs and visceral pain sensation in rodents (D'Argenio et al., 2006; Bashashati et al., 2017). Interestingly, the role of endocannabinoids and biochemically elated bioactive long chain fatty acid derivatives, such as the *N*-acylethanolamines and *N*-acylserotonins, and their targets (defined all together as the endocannabinoidome (Piscitelli et al., 2011; Witkamp, 2016) in the inflammatory and behavioural consequences of microbiota perturbation have never been investigated, despite the fact that these mediators have been clearly implicated in obesity-mediated dysbiosis (Cani et al., 2016), depression-like signs (Navarria et al., 2014; Rubino et al., 2015) and gut inflammatory conditions (Borrelli and Izzo, 2009; Capasso et al., 2014) in experimental models.

In the present study, we used a model of dysbiosis obtained by exposing young healthy mice to a broad-spectrum antimicrobial cocktail (Ampicillin, Streptomycin and Clindamycin, ASC) (Lamouse-Smith et al., 2011), and leading to an imbalanced gut microflora. Moreover, as potent modulator of gut inflammatory/immune-response (D'Inca et al., 2011; Compare et al., 2017), the probiotic Lactobacillus casei DG (LCDG) was used as pharmacological tool to investigate the effects of microbiota changes on both gut and brain. Mice were submitted to a wide range of behavioural, biochemical and electrophysiological tests in vivo and ex vivo to assess whether such microbiota disruption leads to alterations in gut inflammation, depressive-like symptoms, social behaviour and cognitive deficits, changes in brain neuronal firing and microglial-glial activation, or to alterations of select central and peripheral endocannabinodome members. Our findings suggest that probiotic administration may improve microbiota perturbation-associated affective impairments by acting at both central and peripheral levels via several cellular and biochemical mechanisms.

2. Materials and methods

2.1. Animals and treatments

Male C57/bl6 mice (6 weeks) obtained from Jackson Laboratory were housed controlled illumination and environmental conditions for 1 week before the commencement of experiments. The experimental procedures were approved by the Animal Ethics Committee of the Università della Campania of Naples. Animal care was in compliance with the IASP and European Community (E.C. L358/1 18/12/86) guidelines on the use and protection of animals in experimental research. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Ampicillin, Streptomycin and Clindamycin (ASC) were mixed into sterile drinking water (1 mg/ml, for 2 weeks) as previously described (Lamouse-Smith et al., 2011). Drinking solution containing the antibiotics was available for 14 days ad libitum. Control groups of mice drunk water for 14 days. Afterwards control and Dysb mice they were treated via oral gavage with the probiotic (*Lactobacillus casei* DG, 10^9 cells in saline, 100 μl) or saline up to 7 days. Water was replaced every 3 days for the duration of the experiments.

An additional group of mice received saline or ASC via intraperitoneal route (absorbable quote of oral daily doses) for 7 days.

2.2. Behavioural testing

Behavioural tests (N=10–12) were performed on day 14 and 21 from antibiotic exposure (day 0). At the end of each set of experiments mice were sacrificed for further evaluations. The behavioural tests were scheduled in order to avoid carry-over effects from prior testing experience.

2.2.1. Depressive-like behaviour

Tail suspension test (TST). Mice were individually suspended by the tail on a horizontal bar (50 cm from floor) using adhesive tape placed approximately 2 cm from the tip of the tail. The duration of immobility, recorded in seconds, was monitored during the last 4 min of the 6-min test by a time recorder. Immobility time was defined as the absence of escape-oriented behavior. Mice were considered to be immobile when they did not show any body movement, hung passively and completely motionless.

Forced swimming test (FST). Mice were placed in a cylinder (30 cm × 45 cm) filled with water at a temperature of 27 °C, for a 6-min period. The duration of immobility in seconds was monitored during the last 4 min of the 6-min test. Immobility period was defined as the time spent by the animal floating in the water without struggling and making only movements necessary to keep its head above the water. Immediately afterwards, the trial mice were placed under a heating lamp to dry.

2.2.2. Motor coordination behaviour

Possible motor coordination impairment was evaluated by Rotarod test (Ugo Basile). Mice were measured for the time of equilibrium before falling on a rotary cylinder by a magnet that, activated from the fall of the mouse on the plate, allows to record the time of permanence on the cylinder. After a period of adaptation of 30 s, the spin speed gradually increased from 5 to 40 rpm for a maximum time of 5 min. The animals were analyzed by 2 separate tests at 1-h interval in the same day. The time of permanence of the mouse on the cylinder was expressed as latency time (s).