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Gut microbiota modulates alcohol withdrawal-induced anxiety in mice

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ABSTRACT

Excessive alcohol consumption remains a major public health problem that affects millions of people worldwide. Accumulative experimental evidence has suggested an important involvement of gut microbiota in the modulation of host's immunological and neurological functions. However, it is previously unknown whether enteric microbiota is implicated in the formation of alcohol withdrawal-induced anxiety. Using a murine model of chronic alcoholism and withdrawal, we examined the impact of alcohol consumption on the possible alterations of gut microbiota as well as alcohol withdrawal-induced anxiety and behavior changes. The 16S rRNA sequencing revealed that alcohol consumption did not alter the abundance of bacteria, but markedly changed the composition of gut microbiota. Moreover, the transplantation of enteric microbes from alcohol-fed mice to normal healthy controls remarkably shaped the composition of gut bacteria, and elicited behavioral signs of alcohol withdrawal-induced anxiety. Using quantitative real-time polymerase chain reaction, we further confirmed that the expression of genes implicated in alcohol addiction, *BDNF, CRHR1* and *OPRM1,* was also altered by transplantation of gut microbes from alcohol-exposed donors. Collectively, our findings suggested a possibility that the alterations of gut microbiota composition might contribute to the development of alcohol withdrawal-induced anxiety, and reveal potentially new etiologies for treating alcohol addiction.

1. Introduction

According to the World Health Organization, excessive alcohol abuse brings about approximately 3.3 million of deaths in the world each year, and contributes to more than two hundred types of diseases. Individuals who consume alcohol engage in repeated and excessive episodic drinking are considered to be "problem drinkers" (Gorini et al., 2014). In the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM), alcohol abuse and dependence are now gathered in alcohol use disorders (AUDs) that comprise different severity stages (mild, moderate and severe) depending on the number of encountered criterions. AUDs could be identified by alcohol craving, seeking, a loss of control in alcohol consumption as well as alcohol tolerance and withdrawal symptoms, covering anxiety, depressive episodes, social withdrawal, insomnia, nausea and seizures, which can be lethal (Ron and Barak, 2016). A series of genetic (Levey et al., 2014), neurobiological (Levey et al., 2014), environmental (Salvatore et al., 2014) and psychosocial (Whelan et al., 2014) risk factors may contribute to the development of alcoholism, but the molecular mechanisms underlying alcohol addiction remain poorly understood. It has been suggested that long-term alcohol exposure induces changes in microRNA (miRNA), gene and protein expression levels in specific brain regions (Gorini et al., 2014). The expression of several genes, such as BDNF, CRHR1 and OPRM1 have been suggested as possible biomarkers of alcoholism with some diagnostic and prognostic value (Bierut, 2011; Treutlein et al., 2006; Pandey, 2003; Uhl et al., 2001), however, the molecular mechanisms underlying the pathogenesis of alcoholism remain largely unknown. It is thus a major challenge to develop effective therapeutic strategies to clinically manage alcoholism and to alleviate alcohol withdrawal syndrome, partly because of the heterogeneity clinical patient population (Addolorato et al., 2012). Another limitation is that a high level of relapse is observed in patients suffering from AUDs, even after protracted abstinence (Seo et al., 2013). Thus, detailed investigations are urgently needed for better understanding the underlying pathogenic mechanisms of alcoholism and alcohol withdrawal syndrome.

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It is now increasingly recognized that animals share an intimated and life-long partnership with a myriad of resident microbial species, collectively referred to as the microbiota. Gut microbiota governs multiple pathophysiological processes and even regulates the function of distant organs. For instance, intestinal microbes have ascended to prominence as key modulators of host immunity (Belkaid and Hand, 2014), metabolic disease (Moreno-Indias et al., 2014), cardiovascular disease (Aron-Wisnewsky and Clément, 2015), host radiosensitivity (Cui et al., 2017b; Cui et al., 2016) and other diseases (Cui et al., 2017a). There is emerging evidence that the enteric microbiome extends its influence to the brain through various pathways connecting the gut to the central nervous system which formed the gut – microbiota–brain axis (Sampson and Mazmanian, 2015). Moreover, gut microbes impact neurological outcomes altering behavior and potentially affecting the onset and severity of nervous system disorders (Arentsen et al., 2015). Meanwhile, divergent factors, such as genetic predisposition, diet and inflammation states, can differently affect enteric bacterial flora. In this regard, exploring factors that alter the composition and function of gut microbiota are important to understand the processes of alcohol withdrawal-induced anxiety and behavior changes, and to identify new targets for treatment. Heretofore, it is important to assess whether a possible alteration of host gut microbiota contributes to the development of alcohol withdrawal-induced anxiety.

In the present study, we investigate the effect of gut microbiota on the development of alcohol withdrawal-induced anxiety in a rodent model, and obtain that gut microbiota is implicated in alcohol withdrawal-induced anxiety and behavior changes. Our findings suggested a possibility that the alteration of gut microbiota composition may contribute to the development of alcoholism, and revealed potentially therapeutic approaches to mitigate alcohol withdrawal syndrome.

2. Materials and methods

2.1. Animals

Six- to 8-week-old male C57BL/6 mice were kept at the temperature of 21–23 °C under a twelve-hour regular light/dark cycle. Mice were given access to food and water excluding the temporary time of being removed from their cages for conducting the tests. All animals were treated according to the NIH guidelines for use and care of live animals and approved by our Institutional Animal Care and Use Committee (IACUC). All the mice in this study were of a pure C57BL/6 genetic background and separated into groups randomly. All procedures and animals handlings were performed following the ethical guidelines for animal studies.

2.2. Donor stool preparation and administration

The donor's faecal droppings from alcohol-exposed mice (Alcohol group) were collected under SPF conditions. Donor stool was freshly prepared on the day of transplant and that in all cases was prepared and transplanted within 4 h. Donor stool was weighed and diluted with 1 ml of saline per 0.1 g of stool. Briefly, the stool was steeped in saline for about 15 min, shaken and then centrifuged at 800 rpm for 3 min. The supernatant was obtained for transplantation.

2.3. Experimental protocol

The animals were separated into 4 groups: (1) Water group (n = 12): mice were treated with normal water during all experiments; (2) Alcohol group (n = 12): 5% alcoholic solution 0.2 ml was force-fed into the mice's stomach using gavage for one time per day during the first week. Moreover, 5% alcoholic solution was added in their drinking

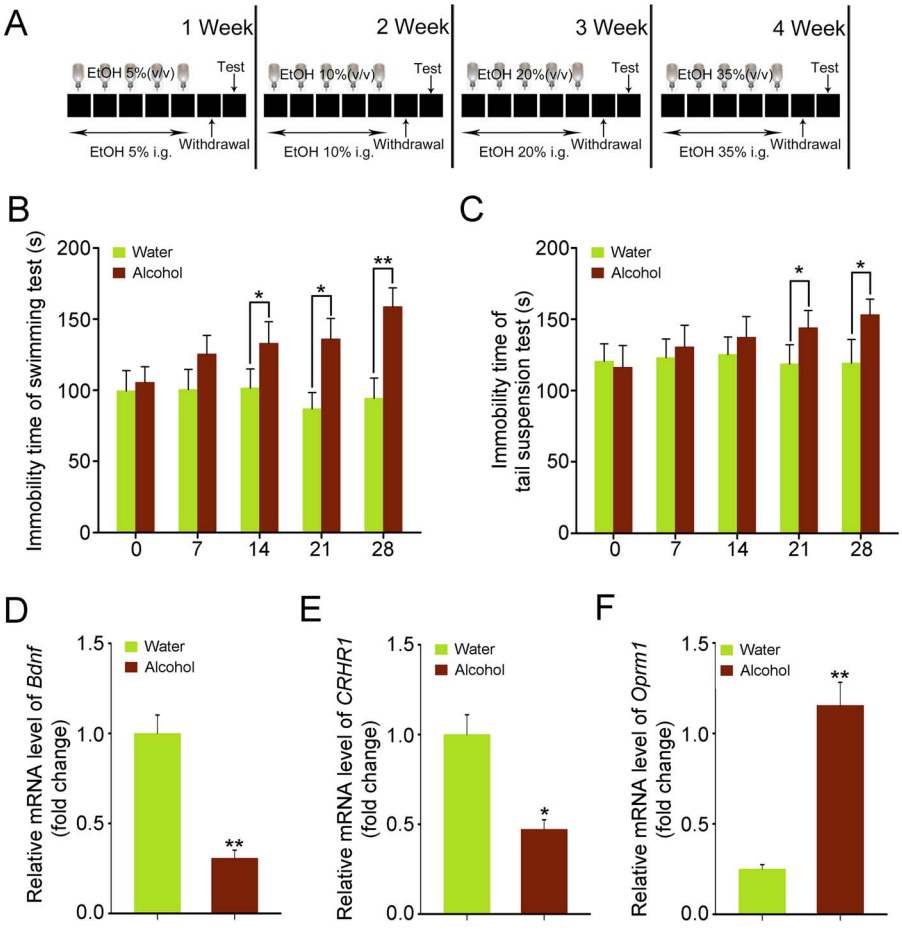

Fig. 1. The successful establishment of murine model of chronic alcoholism. (A) Scheme for experimental protocol. (B, C) The effects of chronic alcohol consumption on cognitive functions. The forced swimming test (B) and tail suspension test (C) were performed at day 7, 14, 21 and 28. (D– F) The expression levels of *Bdnf* (D), *CRHR1* (E) and *Oprm1* (F) in the hippocampus were assessed at day 28 by qRT-PCR, n = 6 per group. Statistically significant differences are indicated: *P < 0.05; **P < 0.01; Student's *t*-test.