Comparison of Microbial Diversity and Composition in the Jejunum and Colon of Alcohol-Dependent Rats

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Introduction

Alcohol, as a common addictive substance, has long been widely consumed worldwide. Long-term alcohol consumption induces alcohol use disorders (AUDs) including alcohol abuse and dependence, which can be identified by alcohol seeking and craving, and alcohol withdrawal symptoms such as anxiety, restlessness, agitation, tremor, seizures, as well as cognitive dysfunction [1, 2]. Alcohol dependence (AD) brings about not only a negative effect on health, but also criminality and hazards to others while imposing an extremely high burden on society [3, 4]. Hence, treating alcohol addiction is certainly an important challenge for the medical community.

Many risk factors may contribute to the development of alcohol dependence including neurobiology [5], environment [6] and psychosocial conditions [7]. However, the formation mechanisms of alcohol dependence have not been fully elucidated to date. The previous studies about the causes of alcohol dependence have mainly focused on...
the effect of alcohol consumption on neuronal functions in the brain. The traditional viewpoint held that ethanol, through the blood-brain barrier, directly or indirectly excites the Ventral Tegmental Area (VTA), increasing the release of some neurotransmitters including dopamine, serotonin, glutamate and γ-aminobutyric acid (GABA), and causing a rewarding effect, which is a pathophysiological foundation of alcohol dependence [8, 9]. However, the drugs used to treat alcoholism target the neurotransmitter systems and have displayed a limited effect, which suggests that there are other possible peripheral biological processes inducing alcohol dependence [10, 11]. Recently, the “brain-gut-microbiota axis” hypothesis has put forth that the gut bacteria may influence brain functions and behavior through many pathways [12, 13]. It is widely accepted that the gut microbiota has important physiological functions and is indispensable for animals and humans alike. It has been proved that intestinal microbiota dysbiosis can cause a variety of somatic diseases including type 2 diabetes [14], allergy [15], inflammatory bowel diseases [16] and obesity [17]. Many documents have also stated that gut microbiota dysbiosis may contribute to some psychiatric disorders such as depression, autism and substance addictions [18–21]. But data about the effects of alcohol dependence on gut microbiota dysbiosis are limited.

Numerous factors are involved in modifying the microbial composition of the gut, including diet, antibiotic use and genetics [22, 23]. Some data also suggest that alcohol and the products of its degradation can strongly disturb the gut microbiota [24, 25]. Bishehsari et al reviewed that chronic alcohol consumption increased gram-negative bacterial overgrowth in the intestine, raised gut permeability and enhanced the plasma levels of gut-derived bacterial products including peptidoglycans and lipopolysaccharides, which contribute to alcoholic hepatitis and can cause injury to other organs [26]. Another study observed that the abundance of Proteobacteria and Actinomycetes increased, but that the normal symbiosis bacteria were reduced after a 3-wk period of alcohol consumption in rats [27]. Although there also have been some studies done on the gut microbiota regarding alcohol dependence and alcoholism, the samples in these studies were from feces instead of the gastrointestinal tract. Moreover, fecal samples are easily, frequently, and continuously collected, and often act as a substitute for microbial communities in the gut. However, that the fecal microbiota represents all the microbial communities in the gastrointestinal tract is inappropriate, and furthermore, the composition of microbiota communities changes with the spatial location within the gut [28]. Some studies in other animal models have demonstrated that microbial communities obviously differ between the gastrointestinal tract and feces as well as between different locations in the gastrointestinal tract [29]. To our knowledge, there has been no literature focusing on gut microbiota variation in different intestinal locations in subjects with alcohol dependence syndrome. Thus, to illuminate further the relationship between gut microbial communities and alcohol dependence syndrome, it is important to reveal the spatial variation in microbiota across the gastrointestinal tract.

In the present study, our aims were to characterize the microbial diversity and composition in the jejunal and colon, and compare the effects of alcohol dependence and alcohol withdrawal on the microbiota of the foregut and hindgut. This study provides stronger evidence for further research on microbial communities and their function in alcohol dependence syndrome.

Materials and Methods

Animals

Adult male Wistar rats weighing 190-210 g at the beginning of the experiments were used in this study. They were purchased from Charles River Laboratories, Beijing, China, with the permission of SCXK (Jing) 2016-0011 (No. 11400700259454). The rats were placed in a quiet room with temperature at (21 ± 2°C) and humidity of (50 ± 5%), in which 12–12h light–dark cycle was maintained (08:00–20:00h light), and the rats were allowed to acclimate to the environment for one week prior to experiment. All animal experimental procedures were approved by the Animal Care and Use Committee of Xinxiang Medical University, China (permission number: AE-2014-09/03).

Alcohol-Dependent Rat Models

The alcohol dependence models were established as described in documents [30]. Briefly, 40 rats were randomly assigned to control and AD groups. Rats were housed in cages (460*300*215 mm), and each group had 4 cages with 5 rats per cage. In AD groups, ethanol was administered in drinking water at gradually increasing concentrations from 1% to 6% (v/v) for the first week, and following this, 6% alcohol was continued during a total of 30 days. Control group rats received tap water only. All liquid was prepared daily and given to the rats at the same time (a.m. 9:00). The weights of the rats were recorded every week, and the daily drinking water (containing alcohol) was measured every day. The alcohol intake of individual animals was expressed as the average value of each cage. At the end of the 30 days, ethanol was withdrawn from the drinking water by providing only tap water until the end of experiments in the AD group.