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Diabetic cognitive dysfunction is associated with increased bile acids in liver and activation of bile acid signaling in intestine

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ABSTRACT

Impaired regulation of bile acid (BA) homeostasis has been suggested to be associated with adverse metabolic consequences. However, whether BA homeostasis is altered in diabetes-induced cognitive dysfunction (DCD) remains unknown. In the present study, mice were divided into four groups, namely normal control (NC) group, high-fat diet (HFD) group, diabetes without cognitive dysfunction (unDCD) group, and DCD group. Compared to HFD mice, the concentration of total BAs in liver was higher in unDCD and DCD mice, due to increased intestinal BA absorption. DCD mice tended to have higher BA concentrations in both liver and ileum than unDCD mice. Consequently, DCD mice had increased basolateral BA efflux (Ostα, Ostβ, and Mrp4) and decreased BA synthesis (Cyp7a1, Cyp8b1, and Cyp7b1) in the liver as well as activated Fxr-Fgf15 signaling in the ileum. DCD mice also had increased BA hydroxylation (Cyp3a11) and BA sulfation (Sult2a1) in the liver compared to HFD mice. Furthermore, the bacterial community composition was altered in the cecum of DCD mice, characterized with a marked increase in *Defferribacteres* and *Candidatus Saccharibacteria*. In summary, the present study provides the first comprehensive analysis of BA homeostasis in DCD mice, and revealed a potential role of BAs in DCD development.

1. Introduction

Bile acids (BAs) are amphipathic molecules that serve as powerful physiological detergents for intestinal absorption of nutrients, fats, and fat-soluble vitamins. BAs are also versatile signaling molecules that regulate various nuclear and membrane receptors, such as farnesoid X receptor (FXR) and G protein coupled receptor TGR5 (Lefebvre et al., 2009; Russell, 2003). Furthermore, BAs regulate calcium mobilization, cyclic AMP synthesis, protein kinase C activation and secretion of pro-inflammatory cytokines (Miyake et al., 2000; Stravitz et al., 1995). It should be noted that individual BAs have distinct structures, and thus have different activities in dissolving cholesterol in bile, promoting nutrient absorption in intestine, activating BA signaling pathways, and causing cytotoxicity in the body. For this reason, the synthesis and metabolism of BAs need to be tightly regulated to achieve their physiological functions.

BAs are appreciated as metabolic integrators and signaling molecules that control lipid, glucose and energy homeostasis (Thomas et al., 2008). Many studies have demonstrated the mutual relations between the onset and progress of diabetes and the homeostasis of BAs. For instance, elevated BA pool and fecal BA excretion were observed in T2DM patients with uncontrolled hyperglycemia (Bennion and Grundy, 1977). T2DM patients had an enlarged pool of secondary BAs, characterized with elevated deoxycholic acid (DCA) in the serum (Suhre et al., 2010). Although the link between BA metabolism and diabetes has been supported by clinical data, the evidence from animal studies is scarce and inconsistent (Prawitt et al., 2011). The discrepancy in animal data is partly due to the use of many diabetic animal models that have different patterns of disease initiation and progression. These models include but not limited to ob/ob mice, db/db mice, KK-Ay mouse, as well as diet- and chemical-induced experimental animal models (Rees and Alcolado, 2005). Additionally, due to the limitation of BA quantification

Abbreviations: BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; DCD, diabetes-induced cognitive dysfunction; HFD, high-fat diet; LCA, lithocholic acid; MCA, muricholic acid; NC, normal control; MDCA, murideoxycholic acid; T-BA, taurine-conjugated bile acid; TC, total cholesterol; TG, triglyceride; TMCA, tauromuricholic acid; U-BA, unconjugated bile acid; UDCA, ursodeoxycholic acid; unDCD, diabetes without cognitive dysfunction

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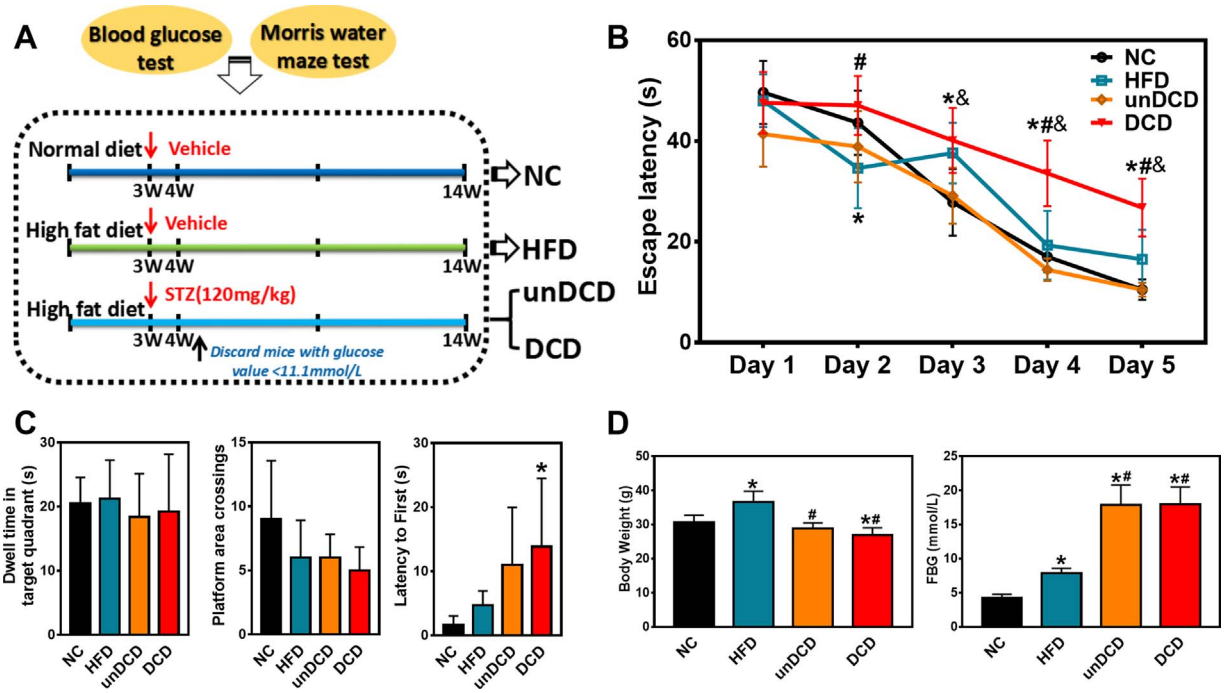

Fig. 1. Establishment and characterization of mouse models with or without DCD. (A) The flow diagram of animal grouping and treatment. Mice were divided into four groups (N = 13–15 per group) to mimic the different stages of DCD. NC, normal control; HFD, high-fat diet; unDCD, diabetes without cognitive dysfunction; DCD, diabetes-induced cognitive dysfunction. (B) Escape latency in the Morris water maze. Data represent means ± S.E.M. (n = 8–10 per group). (C) Percentage of time spent in the target quadrant, frequency of platform corssining, and latency to first quadrant during a 60 s probe trial in the Morris water maze. Data represent means ± S.E.M. (n = 8–10 per group). (D) The body weights and fasting blood glucose levels of mice at the end of the study. Data represent means ± S.E.M. *p < 0.05 versus NC group. #p < 0.05 versus HFD group. &p < 0.05 versus unDCD group.

technique, the information about the alterations in individual BAs during the development of diabetes is quite limited. It has been suggested that a combination of high-fat diet (HFD) and streptozotocin (STZ) could induce a type 2 diabetes model highly analogous to the clinical manifestation in humans (Srinivasan and Ramarao, 2007). However, there is a lack of systematic investigation on individual BAs and genes involved in regulating BA homeostasis.

Diabetes is a systemic disease affecting various organs, such as cardiovascular, gastrointestinal, immune and nervous systems. Strong evidence has suggested adverse effects of diabetes on cognitive system and memory disorders, which is known as diabetes-induced cognitive dysfunction (DCD). Patients with DCD are characterized by cognitive dysfunction accompanied by pathological changes in their brain tissues and will gradually develop dementia (Kodl and Seaquist, 2008). It is estimated that 25–36% diabetic patients develop cognitive impairment, and the incidence of dementia in diabetic patients is 1.5 to 2.5 times that of nondiabetic ones (Quinones and Kaddurah-Daouk, 2009). Although many studies have examined cognitive dysfunction in diabetes patients, more needs to be understood about the mechanisms of DCD in order to develop strategies for prevention and treatment. BAs have been shown to play an essential role in neurological diseases, suggesting an interaction between BAs and the nervous system (Ackerman and Gerhard, 2016; McMillin and DeMorrow, 2016). However, it is unknown about the exact role of BAs in the pathogenesis of DCD.

The present study aimed to evaluate the BA metabolism during the development of DCD and provide the first comparative analysis of BA homeostasis in diabetic mouse models with or without DCD. Adult male C57BL/6 mice were fed either normal chow or a high-fat diet (HFD) with or without a single injection of streptozotocin (STZ). Based on the blood glucose level and behavior test results, mice were divided into four groups, namely normal control (NC) group, HFD group, diabetes without cognitive dysfunction (unDCD) group, and diabetes-induced cognitive dysfunction (DCD) group. The BA concentrations in the liver and intestine, the cecal microbiota, as well as expression of genes involved in BA homeostasis were systematically evaluated.

2. Materials and methods

2.1. Chemicals and reagents

Taurocholic acid (TCA), tauro-β-muricholic acid (TMCA), tauromurideoxycholic acid (TMDCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), taurolithocholic acid (TLCA), cholic acid (CA), α-muricholic acid (αMCA), β-muricholic acid (βMCA), ω-muricholic acid (ωMCA), murideoxycholic acid (MDCA), ursodeoxycholic acid (UDCA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), *iso*-chenodeoxycholic acid (isoCDCA), and *iso*-deoxycholic acid (isoDCA) were purchased from either Sigma-Aldrich (St. Louis, MO, USA) or Steraloids, Inc. (Newport, Rhode Island, USA). Acetonitrile (HPLC grade) was purchased from Fisher Scientific (USA). STZ was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was generated by Q-Gard® 1 Water Purification System (Merck Millipore, Darmstadt, Germany). All other reagents were purchased from commercial vendors and were of the highest purity grade available.

2.2. Animal treatment

Male C57BL/6 mice (12-week-old) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Animals were kept in an environmentally controlled breeding room (temperature: 22 ± 2 °C, humidity: 60 ± 5%, 12 h dark/light cycle). Water and food were provided ad libitum. The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals, and the protocol was approved by the Institutional Animal Care and Use Committee of Tianjin University of Traditional Chinese Medicine (No.TCM-2016-007-E05). The animal model in this study was established according to previous methods (Kusakabe et al., 2009; Mu et al., 2006; Song et al., 2017). The normal control (NC) group was fed a normal diet for 14 weeks and injected once with citrate buffer (vehicle for STZ) during the fourth week. The high-fat diet (HFD) group was fed a high-fat diet