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Chronic rhein treatment improves recognition memory in high-fat diet-induced obese male mice

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Abstract

High-fat (HF) diet modulates gut microbiota and increases plasma concentration of lipopolysaccharide (LPS) which is associated with obesity and its related low-grade inflammation and cognitive decline. Rhein is the main ingredient of the rhubarb plant which has been used as an anti-inflammatory agent for several millennia. However, the potential effects of rhein against HF diet-induced obesity and its associated alteration of gut microbiota, inflammation and cognitive decline have not been studied. In this study, C57BL/6J male mice were fed an HF diet for 8 weeks to induce obesity, and then treated with oral rhein (120 mg/kg body weight/day in HF diet) for a further 6 weeks. Chronic rhein treatment prevented the HF diet-induced recognition memory impairment assessed by the novel object recognition test, neuroinflammation and brain-derived neurotrophic factor (BDNF) deficits in the perirhinal cortex. Furthermore, rhein inhibited the HF diet-induced increased plasma LPS level and the proinflammatory macrophage accumulation in the colon and alteration of microbiota, including decreasing *Bacteroides–Prevotella* spp. and *Desulfovibrios* spp. DNA and increasing *Bifidobacterium* spp. and *Lactobacillus* spp. DNA. Moreover, rhein also reduced body weight and improved glucose tolerance in HF diet-induced obese mice. In conclusion, rhein improved recognition memory and prevented obesity in mice on a chronic HF diet. These beneficial effects occur *via* the modulation of microbiota, hypoendotoxinemia, inhibition of macrophage accumulation, anti-neuroinflammation and the improvement of BDNF expression. Therefore, supplementation with rhein-enriched food or herbal medicine could be beneficial as a preventive strategy for chronic HF diet-induced cognitive decline, microbiota alteration and neuroinflammation.

Keywords: Rhein; Gut microbiota; Recognition memory; Lipopolysaccharide; Inflammation; Perirhinal cortex

1. Introduction

Obesity is a major risk factor for the development of insulin resistance, type 2 diabetes and cognitive decline in neurodegenerative diseases such as Alzheimer's disease (AD) and vascular dementia [1,2]. Patients with AD have been characterized by deficits in recognition memory [3]. The perirhinal cortex plays an important role in higher object recognition memory [4]. Lesions in the perirhinal cortex severely disrupt object recognition [5], object-in-place memory and temporal order recognition memory [6,7] in rodent studies. Empirical evidence has linked high-fat (HF) diet-induced obesity with impairments in learning and memory, including a decline in recognition memory [8] as assessed with the novel object recognition test.

Nowadays, it is widely accepted that obesity and cognitive decline are associated with low-grade systemic and central inflammation, despite the fact that the molecular origin of the inflammation is poorly understood [9,10]. Increased fat intake has been found to be strongly correlated with increased plasma lipopolysaccharide (LPS), endotoxemia [10]. LPS is a major component of the outer membrane in Gram-negative bacteria. Emerging evidence from animal studies suggests a link between the alteration of gut microbiota, increased intestinal permeability and endotoxemia in HF diet-induced obesity [11]. An imbalance of Bacteroidetes and Firmicutes, the primary bacterial phyla comprising the gastrointestinal microbiota, has been reported in rodents fed an HF diet and obese individuals [12,13]. The plasma LPS level was closely correlated with altered intestinal microbiota, in which the number or diversity of the Gram-negative, Bacteroidetes phylum, was

Abbreviations: HF, high fat; LPS, lipopolysaccharide; IPGTT, intraperitoneal glucose tolerance test; BDNF, brain-derived neurotrophic factor; TLR, Toll-like receptor; MyD88, myeloid differentiation primary-response protein 88; JNK, c-Jun N-terminal kinase; NFκB, nuclear factor-kappa B; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

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significantly reduced in animals fed an HF diet [14]. The endogenous LPS is considered to be continuously produced in the gut by the death of Gram-negative bacteria and its translocation into intestinal capillaries *via* the increased intestinal permeability in HF diet-induced obesity [15]. Endotoxinemia in turn can trigger systemic inflammation and neuroinflammation. It has been shown that an intraperitoneal injection of LPS induces neuroinflammation, cognitive impairment and memory dysfunction [16]. LPS binds to Toll-like receptor (TLR) 4 coupled with myeloid differentiation primary-response protein 88 (MyD88)-dependent pathway, and activates c-Jun. N-terminal kinase (JNK) and nuclear factor-kappaB (NFκB), two important inflammatory signaling molecules [17]. The activation of the TLR4–MyD88–JNK/NFκB signaling pathway leads to the production of proinflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and contributes to the development of neurodegenerative diseases [18–21].

Brain-derived neurotrophic factor (BDNF) is known to play an important role in neuronal development and synaptic plasticity in the brain regions involved in cognitive function [22]. In the perirhinal cortex, BDNF has been shown to be important for object recognition memory [23]. Studies have found that BDNF expression in the perirhinal cortex has a positive relationship to recognition memory in rats [23,24], and that treatment with anti-BDNF serum inhibited long-term recognition memory in rats [25]. An intraperitoneal injection of IL-1β or LPS significantly decreases BDNF mRNA expression in the rat hippocampus [26]. Furthermore, the oral administration of antimicrobials in specific-pathogen-free mice transiently altered the composition of the microbiota and increased exploratory behavior and the level of BDNF in the brain [27]. Moderate colonic inflammation induced anxiety-like behavior and decreased BDNF mRNA expression in the brain [28]. Our previous study found that an HF diet impaired recognition memory, decreased BDNF and increased inflammation in the prefrontal cortex of mice [8]. Notably, numerous studies have shown that the beneficial effects of prebiotics and probiotics in obesity occur *via* the modulation of gut microbial homeostasis [29]. Accordingly, the maintenance of a healthy gut microbial environment is important for the treatment of obesity and its related BDNF and cognitive decline.

Rhubarb is usually considered to be a vegetable in Western countries. The dried rhubarb rhizome is an important herbal medicine and has been used for thousands of years. Rhubarb or extract of rhubarb has been reported to possess antibacterial, anti-inflammatory, antioxidative, antidiabetic and neuroprotective properties [30,31]. Rhein (4,5′-dihydroxy-anthraquinone-2-carboxylic acid) is the main ingredient of rhubarb. It has been shown that rhubarb-exposed rats have increased bacterial diversity in the ileum [32]. Several reports have shown that rhein prevents activation of NF-κB and the ERK1/ERK2 pathway [33], and inhibits the synthesis and activity of proinflammatory cytokines [34,35]. Rhein has been reported to be an antibacterial agent which inhibits *Staphylococcus aureus* [36]. Previously, rhein has been reported to decrease body weight gain and fat accumulation in HF diet-induced obese mice [37,38]. However, the potential effects of rhein against alteration of gut microbiota and cognition in HF diet-induced obesity have not been studied. The present study used a chronic HF diet-induced obese mouse model to investigate whether rhein supplementation prevents endotoxinemia, alteration of gut microbiota, recognition memory decline, body weight gain and glucose intolerance in these mice. Furthermore, the neuroinflammatory TLR4–MyD88–JNK/NFκB signaling pathway, proinflammatory cytokines (TNF-α, IL-6 and IL-1β), and neurotrophin BDNF were assessed in the perirhinal cortex.

2. Methods

2.1. Animals and treatments

Twenty-four C57BL/6J male mice (8 weeks old) were obtained from the Australian Bio-Resource Centre (Moss Vale, NSW, Australia) and housed in environmentally controlled conditions (temperature 22 °C, 12-h light/dark cycle). Eight mice were fed a lab chow diet as a control (Con group). Sixteen mice were fed an HF diet (HF group) containing 60% fat by calories (SF13-092; Specialty Feeds, Glen Forrest, WA, USA). After 8 weeks, the 16 mice fed an HF diet were divided into two groups: 8 mice continued to receive the HF diet, and the other 8 mice received the rhein treatment (HF+R group) for 6 weeks. Rhein (0.16%) was mixed in the HF diet (dosage: ~120 mg/kg body weight per day) [39]. Rhein (98%, C15H8O6, MW=284.21) was purchased from Sangon Biotech Co. Ltd., China. Body weight was measured on the last day in every week. Food intake was recorded on the first day in every week. A weighed amount of fresh diet was given at the beginning of the dark cycle. The remaining food in the cage plus spillage was collected and weighed 24 h later. After 6 weeks of treatment, the novel object recognition test and the intraperitoneal glucose tolerance test were carried out. The mice were asphyxiated in chambers prefilled with CO_2 4 days after the tests were carried out. The blood samples were collected into sterile anticoagulant (EDTA) tubes by cardiopuncture using sterile syringes. After the blood samples were centrifuged (10,000*g* for 5 min), the plasma was stored in sterile microcentrifuge tubes at −80 °C. Cecal contents and brain tissue were collected, snap frozen and stored at −80 °C for further analyses as detailed below. The colon tissue was fixed in 10% buffered formalin for immunohistochemistry. The study was approved by the University of Wollongong Animal Ethics Committee (AE13/11), and all animal experiments were conducted in compliance with the National Health and Medical Research Council Australian, Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

2.2. Quantitative real-time polymerase chain reaction to quantify microbial strains from cecal content

The cecal contents of mice were collected immediately after the mice were sacrificed and stored at −80 °C. The QIAamp DNA Stool Minikit (QIAGEN, Germany) was used to extract DNA from cecal contents according to the manufacturer's instructions. Group-specific primers based on 16S rDNA sequences polymerase chain reaction (PCR) assay were forward *Bacteroides–Prevotella*, GAGAGGAAGGTCCCCCAC; reverse *Bacteroides–Prevotella*, CGCTACTTGGCTGGTTCAG; forward *Lactobacillus*, GAGGCAGCAGTAGGGAATCTTC; reverse *Lactobacillus*, GG CCAGTTACTA CCTCTATCCTTCTTC; forward *Bifidobacterium*, CGCGTCTGGTGTGAAAG; reverse *Bifidobacterium*, CCCCACATCCAGCATCCA; forward *Desulfovibrios*, CCGTAGATATCTGGAGGAACATCAG; reverse *Desulfovibrios*, ACATCTA GCATCCATCGTTTACAGC. Quantitative real-time PCR was performed in a 20-μl final reaction volume using a SYBR green I master in a Lightcycler 480 (F. Hoffmann-La Roche Ltd., Switzerland). Amplification was carried out with 40 cycles of 95 °C for 5 s, 60 °C for 10 s and 72 °C for 10 s. Each assay was performed in duplicate in the same run. The level of expression for each gene was calculated using the comparative threshold cycle value (Ct) method, using the formula $2^{-\Delta\Delta Ct}$ (where ΔΔCt=ΔCt sample−ΔCt reference). The final results were expressed as normalized fold values relative to the normal group as described previously [40].

2.3. LPS determination

The concentration of plasma LPS was measured by enzyme-linked immunosorbent assay (LAL assay kit; Hycult Biotech, the Netherlands). The absorbance at 405 nm was measured with a spectrophotometer. A measurable concentration ranges from 0.04 to 10 EU/ml. All samples for LPS measurements were performed in duplicate.

2.4. Immunohistochemistry

The immunohistochemical staining has been described in our previous work [41]. Fixed colon tissues were embedded in paraffin and