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Differential effects of quercetin on hippocampus-dependent learning and memory in mice fed with different diets related with oxidative stress

Shu-Fang Xia, Zhen-Xing Xie, Yi Qiao, Li-Rong Li, Xiang-Rong Cheng, Xue Tang, Yong-Hui Shi, Guo-Wei Le *

The State Key Laboratory of Food Science and Technology, Food Science and Technology, Jiangnan University, Wuxi, China

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ABSTRACT

High fat diets induce oxidative stress which may be involved in neurodegenerative diseases. Quercetin is a kind of antioxidant that has neuroprotective effects and potent7ial pro-oxidant effects as well. In this study, we evaluated cognitive function in mice fed with high fat diets and basic diets with or without quercetin. Male Chinese Kunming (KM) mice were randomly assigned to five groups fed with basic diet (Control), basic diet with 0.005% (w/w) quercetin (CQ1), high fat diet (HFD), HFD with 0.005% (w/w) quercetin (HFDQ1) and 0.01% (w/w) quercetin (HFDQ2) for 13 weeks. At the end of the study period, fasting blood glucose (FBG), plasma and hippocampal markers of oxidative stress, plasma lipid status, Morris water maze as well as hippocampal relative mRNA expression of *akt*, *bdnf*, *camkII*, *creb*, *gsk-3β*, *nrf2* and *pi3k* were examined. The results suggested that in comparison to the control group, the escape latency was increased and percent time spent in the target quadrant was decreased, with increased reactive carbonyls, malondialdehyde (MDA) and declined expression of *pi3k*, *akt*, *nrf2*, *creb* and *bdnf* in the hippocampus of HFD and CQ1 groups. Conversely, higher quercetin supplemented to HFD improved antioxidant capacity and reversed cognitive decline completely. Significant correlations between the redox status and cognition-related gene expression were observed as well ($P < 0.05$). Thus, in the case of oxidative stress, an appropriate dose of quercetin can attenuate oxidative stress to improve hippocampus dependent cognition. But under a balanced situation, quercetin exerts pro-oxidant effects to impair cognition.

1. Introduction

The incidence of obesity is increasing worldwide, especially in developed countries. High-fat diets, rich in saturated fat and monounsaturated fat, are the critical reason for the epidemic. Obesity has been found to be related to myriad diseases, including type 2 diabetes, hypertension, cardiovascular disease, metabolic syndrome, cancer and so on [1]. Nowadays, epidemiological and experimental research has suggested that obesity may be associated with cognitive impairment [2]. Postulated mechanisms include insulin resistance [3], an inflammatory response [1], and oxidative stress [4,5]. Emerging evidence suggests that obesity induced oxidative stress may have an important role in cognition [5]. The brain is especially vulnerable to oxidative stress for its high level of oxygen consumption, a large amount of unsaturated fatty acids, and a relatively deficient antioxidant system and high level of iron which is associated with free radical damage. Attenuating oxidative stress in the brain might consequently be a useful strategy to improve brain function. Therefore, several categories of antioxidants have attracted much attention on the issue.

Antioxidants have been found to exert beneficial effects on neurodegenerative disease [6–8]. A high intake of flavonoids, which contain quercetin, kaempferol, myricetin, luteolin and apigenin, is associated with better cognition at baseline and with a satisfying evolution of cognitive performance over a period of 10 years [9]. Many researchers have focused on the neuroprotective effects of quercetin due to its wide distribution and high concentration in plants. Because quercetin can penetrate the blood–brain barrier even at low concentrations [10], its neuroprotective effects have been attributed to its antioxidant properties and modulation of cell signaling [11]. Although quercetin is an effective free radical scavenger *in vitro* and *in vivo*, some studies indicate that quercetin may have underlying pro-oxidant effects under special conditions [12]. Quercetin is able to auto-oxidize or be transformed to ortho-semiquinone and ortho-quinone/quinone methide intermediates, resulting in the production of reactive oxygen species [13]. Quercetin can be pro-oxidant towards slightly oxidized low-density lipoproteins (LDLs) and at low concentration it can behave as a pro-oxidant towards native LDLs [14]. Although quercetin has been found to improve cognitive impairment induced by D-galactose-treated mice through increasing brain antioxidant capacity [6], whether the application of it on cognition under normal physiological conditions is safe still needs to be clarified.

The hippocampus is the primary region of the brain for spatial learning and memory, as well as for neurogenesis which continues into adulthood, and it is susceptible to the effects of high-fat diets [15]. Previous research has clarified that a high-fat diet can impair hippocampal synaptic plasticity as well as hippocampus-dependent learning and

* Corresponding author. Tel./fax: +86 510 8591 7789.
E-mail address: lgw@jiangnan.edu.cn (G.-W. Le).

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memory [16]. Brain-derived neurotrophic factor (BDNF), a kind of neurotrophic factor, can protect neurons from oxidative stress [17], and can regulate saturated fat-induced activity on hippocampal synaptic plasticity and cognition as well [18]. In contrast, the products of lipid peroxidation derived from oxidative stress could decrease cyclic AMP responsive element binding protein (CREB)-dependent BDNF promoter activity in rat hippocampal neurons [19].

We, therefore, hypothesized that quercetin might have diverse effects under two kinds of diets which represent different physiological conditions. We supplemented basic diets and high-fat diets, respectively, with quercetin and assessed hippocampus-dependent cognition and the antioxidant capacity of the hippocampus as well. We provided novel evidence that dietary supplementation of quercetin improves cognition and that the redox status of the organism should be taken into consideration before using it.

2. Materials and methods

2.1. Animals

Male Chinese Kunming (KM) mice of five weeks old were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China) and housed under conditions of controlled temperature (23 ± 2 °C) and humidity (60%) on a 12-hour light/night cycle. All mice were acclimated to their environment and consumed standard chow for one week. After that, the dietary intervention was started. The experimental protocol was implemented according to the institution's guideline for the care and use of laboratory animals.

2.2. Experimental design

Animals were randomly assigned to five groups (n = 8 animals each group) fed respectively with basic diet (Control, 4.8% fat), basic diet with 0.005% (w/w) quercetin (CQ1, about 8.5 mg/kg body weight of mice) from Sigma Aldrich (≥98% by HPLC, St. Louis, MO, USA), high-fat diet (HFD, 21.2% fat and 1% cholesterol), HFD with 0.005% (w/w) quercetin (HFDQ1) and 0.01% (w/w) quercetin (HFDQ2, about 17 mg/kg). The basic diet is low in saturated fat while the high-fat diet is high in saturated and monounsaturated fat (primarily from lard plus a small amount of soybean oil). The diets contained a standard vitamin and mineral mix with all essential nutrients. All mice had free access to the test diets and purified water throughout the experiment. After the 12-week period, all mice were evaluated in the Morris water maze. After that, the mice were allowed to rest for 1 week and then anesthetized with pentobarbital and sacrificed by decapitation.

2.3. Morris water maze

At 18 weeks of age, the mice were exposed to the Morris water maze (Beijing Sunny Instruments Co. Ltd., Beijing, China) to assess learning and memory performance according to previous procedures [20]. Each mouse received four training periods per day for five consecutive days for acquisition training.

On the sixth day, the probe trial was applied to assess reference memory at the end of learning. For each trial, the mouse was randomly placed in the water facing the pool wall at one of four fixed starting positions, and the time required for each mouse to find the hidden platform during the four 1-min trials was recorded. Once a mouse found the hidden platform, it was allowed to stay on the platform for 15 s, and then returned to its cage for the intertrial interval until all mice had completed the first trial. A mouse that could not find the platform within 1 min was placed on the platform for 15 s at the end of the trial. Acquisition time (latency to find the submerged platform) and the percent of time that a mouse spent on the target quadrant on the last day when the hidden platform was removed were recorded.

2.4. Biochemical analysis

Fasting blood glucose levels were measured using a hand-held glucometer (Johnson & Johnson, New Jersey, USA). The corresponding kits of MDA (determined by the thiobarbituric acid method), catalase (measured by the slightly yellow complexation, produced by the reaction between H_2O_2 and molybdenum-acid-neodymium), superoxide dismutase (SOD, based on its ability to inhibit the oxidation of oxymine by the xanthine–xanthine oxidase system), total antioxidant capacity (TAC, using the ferric reducing ability of plasma method, FRAP) of the left hippocampus and plasma, plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) (all determined by spectrophotometry) were all assessed by the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Reduced glutathione (GSH) and oxidized glutathione (GSSG) were estimated by the procedure of Moron et al. [21]. Total protein contents were determined by means of using bovine serum albumin as a standard by the method of Lowry et al. [22]. ROS in the hippocampus and plasma were measured by a luminol-dependent chemiluminescence assay described by Kobayashi et al. [23], using a MPI-B ultra-weak luminescence analysis system (Xi'an Remix Analysis Instrument Co. Ltd., Xi'an, China). ROS production was expressed as relative light units (RLUs). Carbonyls of proteins were determined immunochemically as adducts of 2,4-dinitrophenylhydrazine method [24].

2.5. Quantitative real-time RT-PCR

Total RNA of the right hippocampus (n = 8 per group) was extracted with a Trizol reagent (Biomiga, San Diego, USA). 1% agarose gel electrophoresis was used to evaluate the quality of the RNA. Total RNA was reverse-transcribed to cDNA according to the manufacturer's instructions (Promega, Madison, Wisconsin, USA). We used Platinum Taq polymerase and SYBR Green I dye (SYBR Green Master Mix, Bioneer, Korea) to measure in the exponential phase of amplification by the ABI PRISM 7500 Sequence Detection System. The primers for the genes are shown in Table 1. The reactions were incubated at 95 °C for 10 min for 1 cycle and then at 95 °C (30 s), 60 °C (45 s), and 72 °C (45 s) for 44 cycles. The final extension was for 5 min at 72 °C. The relative expression levels of the target genes were expressed as a ratio to the housekeeping gene *β-actin*. Meanwhile, melting curve analysis was applied to assess the specificity of the amplified PCR products.

2.6. Statistical analysis

Data are presented as mean ± SD. Statistical analysis was performed by one-way analysis of variance with post-hoc Duncan's test. $P < 0.05$ was considered statistically significant. Pearson's correlation coefficient (r) was used to determine the relation of biochemical markers in the hippocampus to the relative expression of some genes. Analysis was done with SPSS 17 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Effects of quercetin on hippocampus-dependent learning and memory caused by different diets

Our results indicate that mice in the HFD and CQ1 groups had a significant increase in escape latencies compared to the control group, which is in accordance with previous studies ($P < 0.05$). However, quercetin supplementation to the mice fed with HFD alleviated HFD-induced cognitive impairment in a dose-dependent manner (Fig. 1A).

In the probe trial (Fig. 1B), the percent time spent in the target quadrant by HFD and CQ1 groups was significantly decreased ($P < 0.05$ *vs.* Control). The high dose of quercetin supplementation totally reversed a HFD-induced decrease of the retention memory ($P < 0.05$ *vs.* HFD).