Pharmacology, Biochemistry and Behavior 133 (2015) 122–131

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Fibroblast growth factor 21 protects mouse brain against D-galactose induced aging via suppression of oxidative stress response and advanced glycation end products formation

Yinhang Yu [a,1], Fuliang Bai [a,1], Wenfei Wang [a,b], Yaonan Liu [a], Qingyan Yuan [a], Susu Qu [d], Tong Zhang [a], Guiyou Tian [a], Siming Li [a,c], Deshan Li [a,b,*,2], Guiping Ren [a,b,*,2]

[a] Bio-Pharmaceutical Lab, Life Science College, Northeast Agricultural University, Harbin 150030, China
[b] Key Laboratory of Agricultural Biological Function Gene, Harbin 150030, China
[c] Harbin University of Commerce, Harbin 150028, China
[d] Institute of Psychology, Chinese Academy of Sciences, 100101, China

ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 21 March 2015
Accepted 29 March 2015
Available online 11 April 2015

Keywords:
FGF21
D-galactose
Aging
Oxidative stress
AGEs
Inflammation

ABSTRACT

Fibroblast growth factor 21 (FGF21) is a hormone secreted predominantly in the liver, pancreas and adipose tissue. Recently, it has been reported that FGF21-Transgenic mice can extend their lifespan compared with wild type counterparts. Thus, we hypothesize that FGF21 may play some roles in aging of organisms. In this study D-galactose (D-gal)-induced aging mice were used to study the mechanism that FGF21 protects mice from aging. The three-month-old Kunming mice were subcutaneously injected with D-gal (180 $mg \cdot kg^{-1} \cdot d^{-1}$) for 8 weeks and administered simultaneously with FGF21 (1, 2 or 5 $mg \cdot kg^{-1} \cdot d^{-1}$). Our results showed that administration of FGF21 significantly improved behavioral performance of D-gal-treated mice in water maze task and step-down test, reduced brain cell damage in the hippocampus, and attenuated the D-gal-induced production of MDA, ROS and advanced glycation end products (AGEs). At the same time, FGF21 also markedly renewed the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and total anti-oxidation capability (T-AOC), and decreased the enhanced total cholinesterase (TChE) activity in the brain of D-gal-treated mice. The expression of aldose reductase (AR), sorbitol dehydrogenase (SDH) and member-anchored receptor for AGEs (RAGE) declined significantly after FGF21 treatment. Furthermore, FGF21 suppressed inflamm-aging by inhibiting IκBα degradation and NF-κB p65 nuclear translocation. The expression levels of pro-inflammatory cytokines, such as TNF-α and IL-6, decreased significantly. In conclusion, these results suggest that FGF21 protects the aging mice brain from D-gal-induced injury by attenuating oxidative stress damage and decreasing AGE formation.

1. Introduction

Aging is a gradual biological process and induces dysfunction of the normal cells, which affects various systems such as the nervous system and the immune system (Kawakami et al., 1999). Increasing evidence indicates that oxidative stress and advanced glycation end products (AGEs) play very important roles in the process of aging (Halliwell, 2007; Dammann et al., 2012; Rolewska et al., 2012). If the production and elimination of reactive oxygen species (ROS) are not balance, it can cause oxidative stress in the biological system. AGEs are heterogeneous complex group of compounds that are formed when reducing sugar (including glucose, fructose, and hexose-phosphates) and react in a non-enzymatic way with amino acids in proteins, lipids or DNA (Lee and Cerami, 1989). Recently it has reported that excessive forming AGEs can also lead to the accumulation of ROS, inducing the oxidative stress in the body (Yan et al., 2012). Excessive accumulation of ROS and AGES can result in various aging and age-related disorders such as deterioration of learning and memory function and Alzheimer's disease which might be associated with the increasing expression of inflammation related genes (Candela et al., 2010; Shan et al., 2009).

Abbreviations: FGF21, fibroblast growth factor 21; D-gal, D-galactose; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; T-AOC, total anti-oxidation capability; AGEs, advanced glycation end products; TChE, total cholinesterase; AR, aldose reductase; RAGE, member-anchored receptor for AGEs; SDH, sorbitol dehydrogenase; NF-κB p65, nuclear factor-kappa B p65; IκBα, inhibitor of κBα; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6.

* Corresponding authors at: Biopharmaceutical Teaching and Research Section, College of Life Science, Northeast Agricultural University, 59 Mucai Street, Box 180, Harbin 150030, China. Tel./fax: +86 451 59190645.
E-mail addresses: deshanli@163.com (D. Li), renguiping@126.com (G. Ren).

[1] Both Yinhang Yu and Fuliang Bai contributed equally to this study and are co-first authors.

[2] Both Deshan Li and Guiping Ren contributed equally to the design and supervision of the research for this article.

http://dx.doi.org/10.1016/j.pbb.2015.03.020
0091-3057/

As an atypical member of the FGF family, fibroblast growth factor 21 (FGF21) expressing predominantly in pancreas, liver and adipose tissues is identified as a critical regulator of long-term energy balance, glucose and lipid metabolism. FGF21 levels increased in obesity and type 2 diabetes mellitus (Kharitonenkov et al., 2005; Ryden, 2009; Wente et al., 2006; Kharitonenkov et al., 2007; Li et al., 2009). FGF21 can protect human skeletal muscle myotubes from insulin resistance by inhibiting activation of NF-κB (Lee et al., 2012). FGF21 also functions as an endocrine hormone, blocking somatic growth by causing GH resistance, a phenomenon associated with starvation. Besides, Zhang Y. et al. examined the survival time of FGF21-Tg mice and found that chronic FGF21 exposure could markedly extend lifespan in mice, but the mechanism is not fully understood (Zhang et al., 2012).

D-galactose (D-gal) is a reducing sugar and can be metabolized at physiological concentration. However, at high levels, it can be converted into aldose and hydro peroxide which accumulate in cells and react with amines of amino acids in proteins to form advanced glycation end products (AGEs). In addition, excessive accumulation of D-gal in the cell can also result in the generation of reactive oxygen species (ROS) (Song et al., 1999; Ho et al., 2003). The latest reports suggest that AGEs binding to its receptor for advanced glycation end products (RAGE) in many cell types induce pathophysiological cascades linked to the downstream activation of NF-κB and other signaling pathways that lead to ROS generation and certain proinflammatory responses (Mallidis et al., 2007). The long term D-gal rodents have been widely used in recent years for the aging study and drug testing, as D-gal induced behavioral and neurochemical changes and can mimic many characters of the natural brain aging process (Zhang et al., 2004; Cui et al., 2006; Lu et al., 2007; Zhang et al., 2005).

Underline the fact that FGF21-Tg mice significantly extend their lifespan, we speculated that FGF21, as a hormone, may prevent organism from aging. In order to prove the hypothesis we used the D-gal-induced aging model to investigate the roles FGF21 plays for protection of the D-gal mice against brain aging. Our results demonstrate that FGF21 protects the aging mice brain from D-gal-induced injury by attenuating oxidative stress damage and decreasing AGE formation.

2. Materials and methods

2.1. Animals and treatments

Eight-week-old male Kunming mice were purchased from Wei Tong Li Hua Animal Center (Beijing, China). The mice were maintained under constant conditions (23 ± 1 °C and 60% humidity) and had free access to rodent food and tap water. Eight mice were housed per cage on a 12-h light/dark schedule (lights on 08:30–20:30). At 12 weeks of age (37.1 ± 0.6 g), mice were randomly divided into six groups (n = 8 per group), groups 1–4 received daily subcutaneous injection of D-gal (Sigma-Aldrich, MO, USA) at a dose of 180 mg · kg^{-1} · d^{-1} for 8 weeks and group 5 as normal control with injection of saline (0.9%) only. Meanwhile, groups 1–3 D-gal-treated mice received FGF21 of 1 mg · kg^{-1} · d^{-1}, 2 mg · kg^{-1} · d^{-1}, 5 mg · kg^{-1} · d^{-1}, respectively, and group 5 received FGF21 (5 mg · kg^{-1} · d^{-1}) only. All experiments were performed in compliance with the Chinese legislation on the use and care of laboratory animals and were approved by the respective University Committees for Animal Experiments. After the water-maze test and step-down test, mice were sacrificed and brain tissues were immediately collected for experiments or stored at −70 °C for later use.

2.2. Ethological analysis

2.2.1. Water-maze test

A water-maze test, as described previously (Glynn et al., 2003), the apparatus was a 63 cm × 36 cm × 20 cm tank (length × width × height) made of a black rectangular plexiglass tank, of which a number of partitions, and a trapezoidal outlet fixed position. It was divided into five parts which were interlaced and connected, forming a zigzag channel. The water contained was to a depth of 10 cm at 22–24 °C mixed with milk. Mice were selected to ensure their performance at similar level before the treatment of D-gal and FGF21. Each mouse was trained to swim through the zigzag maze from initial region to trapezoidal outlet 5 times per day with 30 s as an interval and arriving at the target (escape latency) in 20 s as a qualified reflection for 4 days. The escape latency and the qualified reflections per mouse each day were recorded.

2.2.2. Step-down test

The apparatus consisted of a plastic box divided into six equal cabins (20 cm × 20 cm × 60 cm high) with grid floor made of stainless steel bar spaced 1 cm apart. The rubber column platform (4 cm in diameter) was fixed in one corner of the cabins. Electric shocks (40 mV) were delivered to the grid floor with an isolated pulse stimulator. Firstly, the animal was placed in the box to adapt for 3 min. Next, electric shocks (1 Hz, 500 ms, 36 DC) were delivered to the grid floor for 5 min. When the mouse stepped down from the platform with all four paws on the grid, the electric shock was delivered, which was counted as an error. After a 24 h interval, the mice were again placed on the platform, the time which elapsed until the mouse stepped down from the platform for the first time (the latency), the number of errors made in 5 min and the total shock time for each mouse was recorded.

2.3. Preparation of tissue samples

Mice were decapitated 60 min after the behavioral tests. The cerebrum was separated on ice, longitudinally bisected along the axes. The left cerebral hemisphere was homogenized with ice-cold saline to be 10% (w/v) homogenate.

2.4. Histological evaluations

For pathological studies, following pre-fixed in 10% methanal for 24 h, the right cerebral hemisphere was postfixed in 70% ethanol for at least 12 h. After dehydration, the brain was embedded in paraffin blocks. Four coronal sections of 2 μm at −4.0 mm posterior from bregma were stained with hematoxylin–eosin. The result was pictured by a Leica camera (Germany).

2.5. Assay of ROS

To measure ROS production, the brain ROS generation was determined in tissue homogenates by using dichlorofluorescein diacetate (DCFH-DA) (Jiancheng Institute of Biotechnology, Nanjing, China) as a probe according to previous literature (Ruan et al., 2013). Briefly, the homogenate was diluted 1:20 (v/v) with PBS buffer (200 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_3, 2.0 mM KH_2PO_3 pH 7.4). The reaction mixture (200 μl) containing 190 μl of homogenate and 10 μl of 1 mM 2′,7′-dichlorodihydrofluorescein diacetate was incubated for 30 min at 37 °C temperature to allow the 2′,7′-dichlorodihydrofluorescein diacetate to be incorporated into any membrane-bound vesicles and the diacetate group to be cleaved by esterases. The conversion of 2′, 7′-dichlorodihydrofluorescein diacetate to the fluorescent product 2′, 7′-dichlorofluorescein was measured using a spectrofluorometer (PerkinElmer, America) with excitation at 484 nm and emission at 530 nm. Background fluorescence (190 μl homogenate, 10 μl PBS absence of 2′,7′-dichlorodihydrofluorescein) was corrected by the inclusion of parallel blanks. ROS formation was quantified from a 2′, 7′-dichlorofluorescein standard curve, and the data are expressed as pmol 2′,7′-dichlorofluorescein formed/min/mg protein.