Neuroscience Letters 571 (2014) 45–49

Contents lists available at ScienceDirect

Neuroscience Letters

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

The influence of gender, age and treatment time on brain oxidative stress and memory impairment induced by D-galactose in mice

Ling Hao[a], Huang Huang[a], Junying Gao[a], Charles Marshall[b], Yali Chen[a], Ming Xiao[a,*]

[a] Jiangsu Province Key Laboratory of Neurodegeneration, Department of Anatomy, Nanjing Medical University, Nanjing 210029, Jiangsu, China
[b] Department of Rehabilitation Sciences, University of Kentucky Center for Excellence in Rural Health, Hazard, KY 41701, USA

HIGHLIGHTS

- Age and treatment time but not gender affect D-gal-induced brain ROS generation.
- Gender, age and treatment time affect D-gal-induced brain oxidative stress.
- Gender, age and treatment time affect D-gal-induced spatial memory deficits.

ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form 22 April 2014
Accepted 24 April 2014
Available online 2 May 2014

Keywords:
D-Galactose
Brain aging
Oxidative stress
Spatial memory
Mice

ABSTRACT

Chronic exposure to D-galactose (D-gal) serves as a model for age-related oxidative damage and cognitive dysfunction. However, methods used, including the dose and treatment time of D-gal as well as the gender, age and strain of animals used, vary greatly among published articles. In this study, we investigate the effect of gender, age and treatment time on brain oxidative stress and spatial memory deficits induced by D-gal in mice, respectively. Eight-week-old female mice injected with 100 mg/kg D-gal per day, for 6 weeks, did not show spatial memory impairment or high levels of hydroxyl radical, protein carbonyl and malondialdehyde in brain homogenates, although brain reactive oxygen species were increased when compared with saline control mice. In contrast, both 8-week-old male mice and 24-week-old female mice receiving 100 mg/kg D-gal for 6 weeks, or 8-week-old female mice receiving 100 mg/kg D-gal for 10 weeks showed spatial memory deficits and significant increases in the above oxidative markers, compared with their corresponding controls. These results demonstrate that D-gal-induced brain oxidative stress and spatial memory impairment are dependent upon exposure time of D-gal, plus gender and age of the animals used. The findings can serve as a useful guide for successfully establishing D-gal induced age-related oxidative damage models.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Oxidative stress is a consequence of an imbalance between pro-oxidant and antioxidant defenses, causing accumulation of reactive oxygen species (ROS). The accumulated ROS lead to oxidative modification of biomolecules such as lipids, proteins and nucleic acids, in turn impairing cellular function and causing cellular senescence [1]. The brain is particularly vulnerable to oxidative damage in part because of its relatively high rate of oxidative metabolic activity, low antioxidant defenses, and high levels of unsaturated fatty acids that are substrates for peroxidation reactions [2]. Therefore, protection against oxidative stress is critical for delaying brain aging and preventing neurodegenerative disorders, such as Alzheimer's disease. In this regard, it would be important to establish suitable animal models for assessing the safety and therapeutic efficacy of brain antioxidants.

D-Galactose (D-gal) is a reducing sugar that can generate ROS during its metabolism in vivo [3]. Rats and mice chronically treated with D-gal show brain oxidative stress and cognitive dysfunction, accompanied with several hallmarks of age-related neurodegeneration such as cholinergic degeneration [4], impairment of synaptic plasticity and neurogenesis [5], altered expression of amyloid-beta metabolism-associated molecules [6], reactive gliosis [7] and neuroinflammation [8]. Therefore, chronic injection of D-gal serves as an animal model for age-related brain oxidative damage and anti-aging pharmacology research.

According to the Pubmed Database of National Library of Medicine, more than 500 papers have been published using D-gal

* Corresponding author. Tel.: +86 25 86862879; fax: +86 25 86862881.
E-mail address: mingx@njmu.edu.cn (M. Xiao).

http://dx.doi.org/10.1016/j.neulet.2014.04.038
0304-3940/© 2014 Elsevier Ireland Ltd. All rights reserved.

model. However, the dose (50–1250 mg/kg) [3,9] and treatment time (14–90 days) [6,10] as well as the animal's gender (female or male) [11,12], age (1–15 months) [13,14] and strain (Kunming, ICR, C57BL/6 J, Swiss albino, etc) [6,10–12] vary in the current literature (for detailed information see electronic Supplementary material). Each of these factors may affect model establishment and therapeutic efficacy of antioxidants. Aside from the dosing effect [15], the influence of other aforementioned factors on D-gal-induced brain oxidative damage remains unclear.

In this study, we investigate the effects of age, gender and treatment time on establishing a mouse D-gal induced aging model. Our results demonstrate that the each factor affects D-gal-induced brain oxidative stress and cognitive impairment.

2. Materials and methods

2.1. Animals and experimental design

C57BL/6 strain mice were housed at 20–25 °C, 60% relative humidity, 12/12-h light/dark cycle, with food and water available ad libitum. Eighty mice were divided into eight groups, with each group ($n = 10$) receiving one of the following treatments (Table 1; for detailed information of animal groups see electronic Supplementary material).

2.2. Y-maze test

On the 43rd day (Group 1–6) or 71st day (Group 7–8), mice were tested for spatial learning and memory using the Y-maze which is designed to examine the innate tendency of mice to explore novelty [12]. The number of arm entries, percentage of entry per arm, and total distance travelled were calculated (For the detailed protocols see electronic Supplementary material).

2.3. Brain tissue homogenate preparation

Immediately after Y-maze test, mice were terminated by decapitation. The hippocampus was promptly dissected and homogenized in ice-cold Locke's buffer to obtain a tissue concentration of 10 mg/ml. The homogenate (10%) was centrifuged at 12,000 × *g* at 4 °C for 10 min and the supernatant was used for biochemical analysis.

2.4. Measurement of ROS in brain homogenates

ROS was measured based on the oxidation of 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) to 2′,7′-dichlorofluorescein (DCF) as described previously [16].

2.5. Measurement of oxidative parameters in brain homogenates

Commercial kits (Nanjing Jiancheng Bioengineering Institute, China) were used to detect hydroxyl radical (HR), protein carbonyl (PC) and malondialdehyde (MDA) levels in brain homogenates. Detailed methods have been described in our previously published report [7].

2.6. Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). Differences among means were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's post hoc tests. $P < 0.05$ was defined as significant.

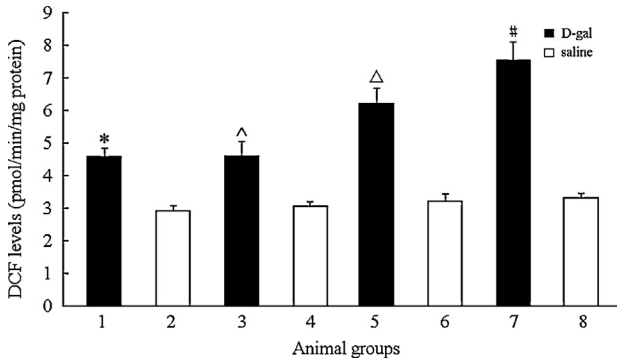

Fig. 1. Analysis of brain ROS levels. Data represent means ± SEM. from six mice per group. * $P < 0.05$, vs. Group 2 and Group 4; ^ $P < 0.05$, vs. Group 2 and Group 4; △ $P < 0.05$, vs. Group 1, Group 2 and Group 6; # $P < 0.05$, vs. Group 1, Group 2 and Group 8.

3. Results

3.1. The effect of gender, age and treatment time on ROS levels in the brain of mice injected D-gal

We first determined the effect of gender on D-gal-induced brain ROS production by comparing levels of DCF fluorescence in the hippocampal homogenate of mice from Group 1, Group 2, Group 3 and Group 4. Two-way ANOVA revealed a significant effect of treatment ($F_{1,20} = 32.497$, $P < 0.001$). Tukey's post hoc tests confirmed that Group 1 and Group 3 had high DCF fluorescence levels compared with Group 2 and Group 4, respectively (all $P < 0.01$; Fig. 1). No effects were observed for gender and treatment-gender interaction ($F_{1,20} = 0.083$, $P = 0.777$; $F_{1,20} = 0.034$, $P = 0.855$, respectively).

We then investigated the effect of age on D-gal-induced brain ROS production by comparing levels of DCF fluorescence among Group 1, Group 2, Group 5 and Group 6. Two-way ANOVA revealed significant effects of treatment, age and treatment-age interaction ($F_{1,20} = 60.081$, $P < 0.001$; $F_{1,20} = 10.676$, $P = 0.004$; $F_{1,20} = 5.026$, $P = 0.036$, respectively). Group 5 exhibited the highest levels of DCF fluorescence ($P < 0.001$, vs Group 1; $P < 0.01$, vs Group 1; $P < 0.001$, vs Group 6, respectively; Fig. 1). Group 1 also had higher DCF fluorescence levels than Group 2 and Group 6 ($P < 0.01$; $P < 0.05$, respectively). There was no difference between Group 2 and Group 6 ($P > 0.05$).

Finally, we examined the effect of exposure time on D-gal-induced brain ROS production among Group 1, Group 2, Group 7 and Group 8. Two-way ANOVA revealed significant effects of treatment, time and treatment-time interaction ($F_{1,20} = 79.786$, $P < 0.001$; $F_{1,20} = 26.094$, $P < 0.001$; $F_{1,20} = 15.158$, $P = 0.001$, respectively). Group 7 had highest DCF fluorescence levels ($P < 0.001$, vs Group 1, Group 2 or Group 8; Fig. 1). There was no difference between Group 8 and Group 1 or Group 2 (both $P > 0.05$).

3.2. The effect of gender, age and treatment time on D-gal-induced brain oxidative stress

We further addressed whether D-gal-induced ROS production resulted in brain oxidative stress, and could be affected by gender, age or treatment time. The gender effect was investigated by comparing levels of HR, PC and MDA levels, markers of oxidative damage in DNA, proteins and lipids in the hippocampal homogenate of mice from Group 1, Group 2, Group 3 and Group 4. Two-way ANOVA revealed significant effects of treatment, gender and treatment-gender interaction on the levels of HR ($F_{1,20} = 5.802$, $P = 0.026$; $F_{1,20} = 50.735$, $P < 0.001$; $F_{1,20} = 14.402$, $P = 0.048$, respectively), PC ($F_{1,20} = 10.435$, $P = 0.004$;

Download English Version:

https://daneshyari.com/en/article/6281806

Download Persian Version:

https://daneshyari.com/article/6281806

Daneshyari.com