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Intranasal Cerebrolysin Attenuates Learning and Memory Impairments in D-galactose-Induced Senescence in Mice

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ABSTRACT

Neurotrophic factors are currently being considered as pro-cognitive therapeutic approaches for management of cognitive deficits. This study aims to evaluate the effects of intranasal (i.n.) or intraperitoneal (i.p.) administration of Cerebrolysin (CBL) (as a mixture of neurotrophic factors) on the d-galactose-induced oxidative stress, apoptosis and memory as well as learning impairment in mice.

For this purpose, CBL (1, 2.5, 5 ml/kg/i.p.) or (1 ml/kg/i.n.), were administrated daily in d-galactose-received (100 mg/kg/subcutaneous (s.c.)) mice model of aging for eight weeks. Spatial and recognition memories were assessed by the Morris water maze and novel object recognition tasks. Brain and blood of animals were analysed for oxidative stress biomarkers including malondialdehyde, total antioxidant capacity, glutathione peroxidase and superoxide dismutase. Apoptosis rate in the hippocampus was evaluated by TUNEL staining of brain tissue. 5 ml/kg/i.p. dose of CBL increased the locomotor activity but, 1 ml/kg/i.p. dose didn't show detectable behavioural or molecular effects on aged mice. Treatment with 2.5 ml/kg/i.p. and 1 ml/kg/i.n. doses attenuated d-galactose-impaired spatial and recognition memories. Results showed an obvious increase in the antioxidant biomarkers and decrease in the malondialdehyde levels both in the blood and brain of aged mice in 2.5 ml/kg/i.p. dose, and only in the brain in 1 ml/kg/i.n. dose of CBL. Anti-apoptotic effects also were seen in the same dose/rout of CBL administration in aged animals.

This study proves the usefulness of i.n. CBL administration as a non-invasive and efficient method of drug delivery to the brain to improve aging-induced oxidative stress, apoptosis and learning as well as memory impairment.

1. Introduction

Ageing is a progressive accumulation of biological changes through time and a multidimensional process in which progressive loss of physiological integrity, is associated with or responsible for susceptibility to disease and decreased quality of life (López-Otín et al., 2013; Lu et al., 2010b). Brain ageing is the cornerstone of ageing which is demonstrated by behavioural deficits such as cognitive decline. Evidence shows that molecular changes including increased oxidative stress and apoptosis participate in aging-related cognitive impairments (Chakrabarti et al., 2014; Mora, 2013).

D-galactose mimics many behavioural and molecular features of brain ageing in rodent models thus, it has been used to investigate the mechanisms of brain ageing and anti-aging therapeutics in animal models (Hsieh et al., 2009; Li et al., 2015; Wei et al., 2005). From the behavioural view, d-galactose induces spatial and recognition memories impairment in rodents (Nam et al., 2013; Yoo et al., 2012). On the other hand, mounting evidence shows that chronic systemic injection of d-galactose increases oxidative stress and its downstream consequences (Cui et al., 2006; Lei et al., 2008; Zhang et al., 2009). Further, high concentrations of d-galactose change into galacticol which accumulates in the cell and generates reactive oxygen spices (ROS). It also increases malondialdehyde (MDA) level and total antioxidant capacity (TAC) and decreases the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-px), monoamine oxidase (MAO) B as well as catalase, all of which increase oxidative stress (Guan et al., 2015; Ho et al., 2003; Lu et al., 2010b; Turgut et al., 2015). In addition, d-galactose causes apoptosis, an important role player in the brain ageing, through activation of NF-kB pathway and increase in protein production of Bax and caspase-3 (Tsai and Yin, 2012). Evidence indicates that both increased oxidative damage and apoptosis may significantly contribute to the development of early cognitive dysfunction in ageing (Lee and Wei, 2007; Zhang et al., 2012).

Brain trophic factors are a group of molecules that are involved in the brain repair. They include but not limited to nerve growth factor (NGF), basic fibroblast growth factor (bFGF) and brain-derived neurotrophic factor (BDNF). They are involved in axonal growth, improvement of cerebral perfusion and cholinergic function as well as neurogenesis (Gutiérrez-Fernández et al., 2012). They have been proposed as treatment strategies for cognitive impairment (Bartus et al., 2013; Bartus and Johnson, 2016;

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Schapira et al., 2014). Cerebrolysin (CBL) is a complex lipid free mixture of peptides and amino acids which act as neurotrophic factors. It is derived from porcine brain proteins and contains the glial-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), NGF and, BDNF (Kumaran Menon et al., 2012). It has been found to improve cognitive functions in patients with ischemic stroke and neurodegenerative disorders (Molloy and Standish, 2000; Ubhi et al., 2013). According to the literature, it appears that administration of CBL reduces both oxidative stress and apoptosis in the brain (Antón and Fuentes, 2011; Formichi et al., 2012).

The intranasal (i.n.) route of administration provides an alternative to other delivery routes of drugs both in animal models and human being (De Rosa et al., 2005; Farzampour et al., 2016). It is a non-invasive route of brain targeting that bypasses hepatic first-pass metabolism and blood brain barrier, uses the olfactory region as a direct and fast connection between the nose and the brain and nowadays is gaining increasing interest (Serralheiro et al., 2014; Xiao et al., 2013).

This study aims to evaluate the effects of i.n. or intraperitoneal (i.p.) administration of CBL on the d-galactose-induced memory and learning impairment in mice.

2. Materials and methods

2.1. Animals

Ninety male BALB/c mice aged 8 weeks and weighing 25–30 g were obtained from Tabriz University of Medical Sciences laboratory animal facility. Animals were housed in controlled environmental condition (12/12-h light/dark cycle starting at 7:00 AM and temperature of 25 ± 2 °C) and standard polypropylene cages (five in each cage). Before and through study the access to tap water and standard pellet food was provided *ad libitum*.

All of the experimental procedures were conducted in conformity with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH; Publication No. 85-23, revised 1985) and approved by the regional ethics committee of Tabriz University of Medical Sciences (No: 1395.577). All efforts were made to minimise animal suffering and the number of animals used.

2.2. Study Design

The animals were randomised into the control (n = 15) and aged groups (d-galactose-received animals) (n = 75). The control group received normal saline (0.9% NaCl) (0.2 ml/animal) and the aged group received d-galactose (100 mg/kg/daily) through subcutaneous (s.c.) route for eight weeks (Prakash and Kumar, 2013). Separate groups of d-galactose-injected mice received normal saline or CBL (215.2 mg/1 mL; EVER Neuro Pharma GmbH, Unterach, Austria) through i.p. (1, 2.5, 5 ml/kg) or i.n. (1 ml/kg) routes, drops containing 5-6 μl of CBL were administered nasally with alternation between right and left nares every two minutes up to total desired volume (Hanson et al., 2009), once daily for eight weeks (Fig. 1). All of the allocations were concealed i.e., the induction of ageing model was blinded.

2.3. Novel object recognition (NOR) test

NOR was conducted in 3 consecutive stages including habituation, training and retention phases. Plexiglas open-field box (33 × 33 × 20 cm) and common objects were used for the test. Animal nose direction to the object (distance ≤ 2cm) and rearing up against the object were considered as exploration. The arena and objects were cleaned with a 70% ethanol solution following each trial. A video camera fixed above the centre of the task apparatus was used to obtain the data. The total locomotor activity of the animal in the habituation session and the time spent for each object in the training and retention phases were recorded and scored using fully automated EthoVision XT video tracking software.

The habituation session started one day before the training step. Each mouse was placed in the box for habituation for 10 min, in the absence of objects and then, locomotor activity was recorded through the session. During the training session which starts one day after the habituation session, two identical objects (A and A') were placed in the box and the total time (up to twenty seconds) spent to explore both objects was recorded over 10 minutes. Retention session was performed the next day after the training trial. The animals were returned to the same task, but one of the familiar objects applied during the training session was replaced by a novel object (B). To evaluate task acquisition

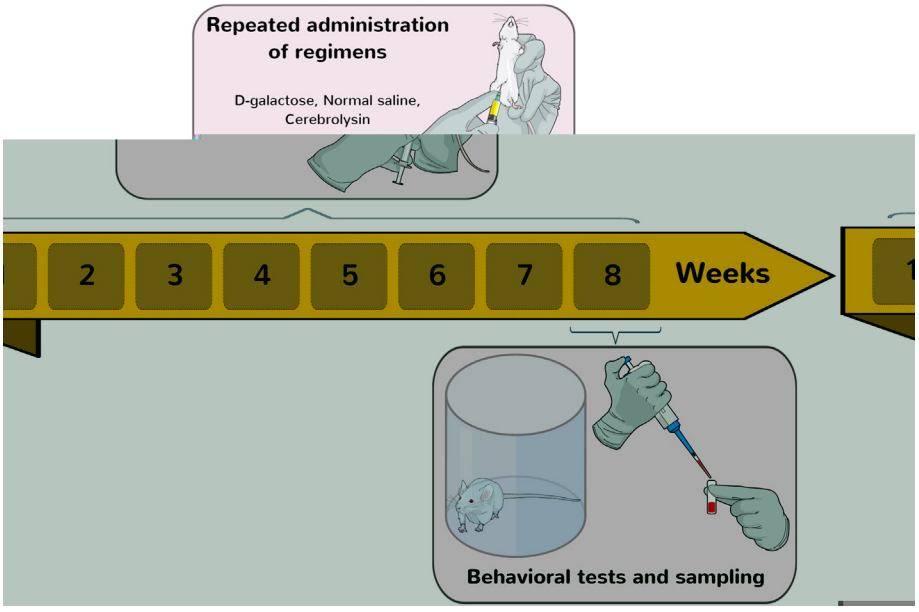

Fig. 1. Treatments administration timescale, behavioural tests and sampling. The figure was created in the Mind the Graph platform, www.mindthegraph.com.