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Simvastatin enhances the hippocampal klotho in a rat model of streptozotocin-induced cognitive decline

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ABSTRACT

Brain oxidative status is a crucial factor in the development of sporadic Alzheimer's disease (AD). Klotho, an anti-aging protein, diminishes oxidative stress by the induction of manganese superoxide dismutase (MnSOD). Thus, the substances that increase klotho expression could be considered as a potential treatment for Alzheimer's disease when the oxidative imbalance is present. Statins are suggested to up-regulate klotho expression. We examined the effect of simvastatin (5 mg/kg, daily for 3 weeks) on hippocampal klotho and MnSOD expression in the cognitive declined animal model induced by intracerebroventricular (ICV)-streptozotocin (STZ) administration. Cognitive assessment was performed by the Morris Water Maze (MWM) test. The results indicated that mean escape latency and distance were prolonged in the ICV-STZ group compared with the control group. The expression of klotho and MnSOD were also down regulated in the hippocampus. Furthermore, improved spatial performance was observed in simvastatin-treated animals. This effect could be related to increase in oxidative stress tolerance as evidenced by klotho and MnSOD up-regulation. Our current study indicates that klotho upregulation may be a neuroprotective mechanism of simvastatin against cognitive decline in AD.

1. Introduction

Alzheimer's disease (AD) is a critical problem with the aging of population which is characterized by the presence of amyloid plaques, and neurofibrillary tangles, resulting in cognitive decline (Tanzi and Bertram, 2005). Neurodegenerative diseases have multifactorial feature which complicates the treatment. Oxidative imbalance may be one of many possible causes of AD (Bhat et al., 2015). As well as brain tissue is susceptible to oxidative stress (Calabrese et al., 2007; Dror et al., 2014). Moreover, the studies indicate that the brain oxidative damage and reactive oxygen species (ROS) generation are involved in the AD development (Lin and Beal, 2006; Manolopoulos et al., 2010).

Klotho, an anti-aging protein, which is expressed by hippocampal neurons (Kuro-o et al., 1997; Shiozaki et al., 2008), plays an antioxidative role and controls redox homeostasis (Yamamoto et al., 2005). Klotho-deficient mice show hippocampal neurodegeneration (Li et al., 2004;Shiozaki et al., 2008) and memory deficits (Nagai et al., 2003). Several studies have revealed that klotho diminishes oxidative stress by inducing the transcription of antioxidative enzymes, including manganese superoxide dismutase (MnSOD) (Utsugi et al., 2000; Yamamoto et al., 2005). In addition, down regulation of klotho expression has been suggested in AD patients (Richard et al., 2014). Therefore, substances that upregulate klotho expression could be considered as therapeutic tools for the treatment of memory deficits associated with AD (Abraham et al., 2012; King et al., 2012).

Several studies have demonstrated that statins have antioxidant properties (Bandoh et al., 2000; Piermartiri et al., 2010; Tramontina et al., 2011; Vaughan and Delanty, 1999). In addition, the neuroprotective role of statins, (Douma et al., 2011; Sharma et al., 2008; Wolozin et al., 2007;Zipp et al., 2007), are also reported. However, the mechanisms underlying this function are only partly understood. There is also a report of statin effect on renal klotho up-regulation (Yoon et al., 2012). Whether the neuroprotective effect of statins is mediated by klotho regulation is not known. In this regard, we assessed the effect of simvastatin on hippocampal klotho and MnSOD expression in an animal model of cognitive decline. Induction of cognitive impairment was performed with

Abbreviations: AD, Alzheimer's disease; DMSO, dimethyl sulfoxide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ICV-STZ, intracerebroventricular-streptozotocin; MWM, Morris water maze; RT-PCR, reverse transcription polymerase chain reaction.

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the intracerebroventricular (ICV)-streptozotocin (STZ) infusion (Lannert and Hoyer, 1998; Sharma and Gupta, 2001). The ICV-STZ injection, decreases the tyrosine kinase activity of insulin receptor, and causes glycogen synthase kinase activation. Ultimately, stimulates tau phosphorylation, and results in neuronal and synapse dysfunction similar to AD pathology (Deng et al., 2009). According to the previous studies, cognitive impairment was evaluated three weeks after ICV-STZ administration (Adel Ghahraman et al., 2016; Hosseinzadeh et al., 2013, 2015) with Morris Water Maze (MWM) test. The hippocampal klotho and MnSOD expression were assessed by real-time RT-PCR and immunohistochemistry. Hippocampal morphological changes were investigated with cresyl violet staining and cholesterol content of serum were also measured.

2. Materials and methods

2.1. Animals

Study was performed on male Wistar rats weighing 280–290 g which were maintained in a controlled room with the temperature of 22 ± 2 °C, and 12-h light/dark cycle whiles received food and water ad libitum. Experiments carried out according to the Guidelines for Care and Use of Laboratory Animals and were approved by the Research and Ethics Committee of the University.

2.2. Stereotaxic surgery

For stereotaxic surgery, anesthesia was induced with ketamine (60 mg/kg, ip; Alfasan, Netherlands) and xylazine (15 mg/kg, ip; Alfasan, Woerden, Holland). Animals were placed on stereotaxic frame (Stoelting Inc., USA) and a guide cannula were inserted toward the lateral ventricles using the coordinates of 1.5 mm lateral to sagittal suture; 0.8 mm back to Bergman; and 4 mm below the surface of brain (Paxinos and Watson, 1986). A heating pad was used during surgery, to maintain body temperature at 36 ± 0.5 °C. Following fixation of guide cannula with dental cement, ampicillin (50 mg/kg, intramuscular, single dose) was injected. Implantation of cannula was performed for a total of 56 rats, but only 50 animals were recovered and included in the following experiments.

2.3. Drug administration

After one-week recovery, open field test was performed. Animals' inclusion in the experiments was according to the normal locomotor activity and general health which was demonstrated by weight gain. ICV–STZ (3 mg/kg, 1.5 mg/5 μl/side,0.4 μl/min, Alexis, Lausen, Switzerland) or saline (0.4 μl/min, 5 μl/site) were infused on days 1 and 3 through a Hamilton syringe connected to a NE-1000 pump (Farmingdale, NY, USA). Simvastatin (ab120505; Cambridge, UK) was dissolved in sterile water containing 10% dimethyl sulfoxide (DMSO) and administered with oral gavage for 21 days. A schematic image of the procedure and time scales for drug administration is shown in Fig. 1.

2.4. Experimental groups

50 male rats were randomly distributed into five groups. All groups experienced stereotaxic surgery except for the control group (Fig. 1). Animals in (ICV-Saline) + DMSO group received bilateral ICV infusion of saline (STZ vehicle, 0.4 μl/min, on days 1 & 3), andoral gavage of DMSO (simvastatin vehicle, daily, for 21 days).

In (ICV-STZ) + DMSO group, animals received bilateral ICV infusion of STZ (3 mg/kg, 1.5 mg/site, on days 1 & 3) and oral gavage of DMSO (daily, for 21 days). Animals in (ICV-STZ) + Sim group received bilateral ICV infusion of STZ (3 mg/kg, 1.5 mg/site) and oral gavage of simvastatin (5 mg/kg, daily, for 21 days). In (ICV-saline) + Sim group, rats received bilateral ICV infusion of saline (0.4 μl/min, on days 1 & 3) and oral gavage of simvastatin (5 mg/kg, daily, for 21 days).

2.5. Behavioral tests

Behavioral tests were carried out between 10:00 and 13:00 in a silent experimental room. The spontaneous motor activity was checked for 5 min as described by Brown et al. (1999). The ccriteria for locomotor activity were the number of line crosses and the frequency of rearing.

2.6. Morris water maze test

The MWM test was started on the day 17 (Fig. 1). A black circular water tank with the 140 cm diameter, 60 cm high, 30 cm depth, and water temperature of 28 ± 1 °C in a silent room with a number of spatial clues was used. Movements of rats were monitored and recorded via a hanging digital video camera (Software: Radiab1 v2 equipment, Iran) from the ceiling above the tank. Four equal quadrants were considered in the pool. The start position was selected randomly and if a rat could not find the platform in 60 s, the examiner put the animal on the platform for 5 s. At first day, 4 trials with visible platform were performed. On the three subsequent days, 4 trials in a day were performed while the platform was invisible and submerged below the surface of the fluid. The ceiling time was 60 s and the trial interval was set at 30 s. The escape latency and the swimming distance were recorded as the time taken and distance moved to reach the platform. The day after the last training session, the platform was removed and probe trial was performed. In probe trial, the time and distance that each rat spent in the targeted quadrant which had previously located the hidden platform, were recorded (Morris, 1984).

2.7. Tissue and blood collection

At the end of experiments, rats were anesthetized with ketamine, xylazine and blood samples were obtained. Serum was prepared and stored at −80 °C until use. For the histopathological examinations, 4 rats from each group were perfused with phosphate buffered saline (PBS) and subsequently 4% paraformaldehyde via the ascending aorta, and brains were removed for immunohistochemistry and cresyl violet staining. Brains of 6 rats of each group were stored in RNAlater reagent (Ambion, Austin, Texas, USA) until further analysis.

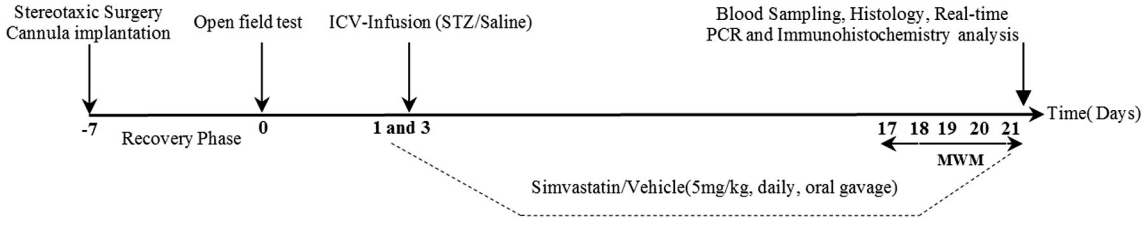

Fig. 1. A schematic image of time scales of the experimental design.