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Streptozotocin-induced hippocampal astrogliosis and insulin signaling malfunction as experimental scales for subclinical sporadic Alzheimer model

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ABSTRACT

Aims: Insulin signaling malfunction has recently been suggested as a preliminary event involved in the etiology of Sporadic Alzheimer's disease (SAD). In order to develop insulin resistance-related SAD model, rats were treated with streptozotocin, intracerebroventricularly (icv-STZ). Nevertheless, given the lack of knowledge regarding sub-clinical stages of SAD, the current challenging issue is establishing a practical pre-clinical SAD model. Despite some proposed mechanisms, such as insulin malfunction, neuroinflammation, and gliosis, icv-STZ mechanism of action is not fully understood yet and Streptozotocin-induced rat model of Alzheimer has still major shortcomings.

Main methods: Using three STZ doses (0.5, 1, and 3 mg/kg) and three testing time (short-term, medium-term and long-term), we sought the best dose of STZ in order to mimic the characteristic feature of sAD in rats. So, we conducted a series of fifteen-week follow-up cognitive and non-cognitive studies. Besides, IR, tau and ChAT mRNA levels were measured, along with histological analysis of astrocyte, dark neuron numbers, and pyramidal layer thickness, in order to compare the effects of different doses of icv-STZ.

Key findings: STZ 3 mg/kg caused cognitive and insulin signaling disturbance from the very first testing-time. STZ1-injected animals, however, showed an augmented hippocampal astrocyte numbers in a short time; they, also, were diagnosed with disturbed insulin signaling in medium-term post icv-STZ-injection. Moreover, behavioral, molecular and histological impairments induced by 0.5 mg/kg icv-STZ were slowly progressing in comparison to high doses of STZ.

Significance: STZ1 and 0.5 mg/kg-treated animals are, respectively, suggested as a suitable experimental model of MCI, and sub-clinical stage.

1. Introduction

A growing body of evidence has indicated that sAD is multifactorial, with age being an important risk factor. Main neuropathological hallmarks identified in Alzheimer's brains are beta-amyloid deposits, hyperphosphorylated tau proteins, and astrogliosis [1]. Recently, dysregulated brain insulin signaling has been reported to play a pivotal role in AD pathology. Additionally, several studies have indicated that insulin malfunction induces beta amyloid accumulation and tau pathology in AD brain [2]. Thus, Alzheimer's disease has been argued to be a type III diabetes [3,4]. Given the specific nature of sAD, there is no clinical evidence on its early stage, and this makes making an appropriate model more difficult. Thus, a convenient model for the initiation phase of sAD could be the beginning step to explore the mechanisms involved in the pathology of Alzheimer disease. Several animal models have been established among which icv-STZ model (icv-STZ administration in rat) has been foregrounded as a hopefully suitable model to investigate the mechanism of sAD progression and explore the neuroprotective potential of some components up to now [5,6]. Importantly, this model could mimic some features of sporadic AD, such as disturbance in some aspects of learning and memory, beta-amyloid accumulation, hyperphosphorylation of tau protein (microtubule-associated protein tau, MAPT) and dysregulation of insulin-related gene expression (for instance, insulin, insulin receptor (IR), choline acetyltransferase (ChAT), and MAPT). In this regard, due to some data proposing insulin resistance as a possible mechanism of ip-STZ, and

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regarding the presence of insulin receptor in rat brain, it has been suggested that icv-STZ injection could induce central insulin resistance, but this mechanism has not been supported by the experimental data yet [6]. Also, Oxidative stress and subsequent neuroinflammation leading to the neuronal death is the second proposed icv-STZ mechanism [7,8]. With respect to the aforementioned evidence, sAD is a chronic and slowly progressing disease. However, STZ doses used in the present model did not turn out to, desirably, imitate the development and progression of AD pathologic features. Furthermore, almost all icv-STZ–based studies have ultimately presented first month post-icv-STZ injection as a suitable testing time. These limitations encouraged us to seek an appropriate dose and testing time of icv-STZ model in order to observe more similar progression in cognitive decline, along with the molecular and neuropathological characteristic features of sAD of slowly progressive nature. Up to now, researches have not comprehensively investigated up to 15-week post-injection behavioral, molecular and neuropathological effects of different doses of icv-STZ. In this regard, the effects of three distinct doses of icv-STZ (0.5, 1 and 3 mg/kg) were compared on these features in three post-injection time points, including short-term (3–5-weeks), medium-term (9–11 weeks) and long-term (13–15 weeks) effects.

2. Materials and methods

2.1. Animals and surgical procedures

Male Wistar rats (3 months old, weighing 280-300 g) were used throughout this study. Rats were housed in groups of 3–4 and kept under standard conditions of temperature (22 °C ± 2) and humidity (40–50%). A 12-h light/dark cycle was provided and animals had ad libitum access to food and water. For icv injection, the animals were anesthetized by i.p. injection of ketamine-xylazine mixture (100 mg/kg–10 mg/kg, respectively) and fixed in a stereotaxic apparatus. A single injection of STZ (0.5, 1 and 3 mg/kg) or saline was applied into the right lateral ventricle (0.8 mm posterior to bregma, 1.5 mm lateral to the sagittal suture, and 3.6 mm beneath the brain surface). Injection volume was 6 μl. The experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1978), and were approved by the Ferdowsi University of Mashhad ethical review process.

2.1.1. Experimental setup

Group 1. Control: Rats undergone stereotaxic surgery and received icv-STZ vehicle (saline).

Group 2. STZ 0.5: Rats received STZ (0.5 mg/kg) on the 1st day.

Group 3. STZ1: Rats received icv-STZ (1 mg/kg) on the 1st day.

Group 4. STZ 3: Rats received icv-STZ (3 mg/kg) on the 1st day.

The animals were subjected to a battery of behavioral tests during 3–5, 9–11, and 13–15 weeks following icv injection. The behavioral studies included Accelerating Rotarod, Y Maze, and novel object recognition task (NORT) accompanying with Morris water maze. All observations were performed between 10.00 am and 16.00 pm. Equipment was cleaned with 70% ethanol between each test to eliminate olfactory cues. After every test battery, ten animals from each group were sacrificed by decapitation (Fig. 1), and their brains were removed quickly. Five brains were kept in 4% paraformaldehyde for 24 h and were then paraffin-embedded for histological studies on the hippocampus (hematoxylin and eosin and Congo-red staining). The hippocampi of the other brains were dissected and stored in − 70 °C for Real-time RT-PCR quantification of insulin receptor (IR), tau and choline acetyltransferase (ChAT).

2.2. Behavioral tests

2.2.1. Accelerating rotarod

In the first part of this study, short-term, medium-term and the long-term effects of icv-STZ injection on the rotarod performance were measured using an accelerating rotarod device (4–40 rpm Rota-Rod 7650; Ugo Basile, Comerio, Italy) in order to prove that the animal motor coordination was not affected by intraventricular drug injection and icv-STZ, per se, had no toxic effect on the animal's motor balance over the time; this was performed once and the mean delay time of the first falling off a rotating rod, known as fall latency, was calculated in each group. Animals were individually placed on a rod facing opposite to the direction of rotation. Each animal was placed on the rod rotating at a constant speed of 4 rpm and trained to remain balanced during training. One session of five trials (180 s each) was given, as described elsewhere, during the retention phase [9]. Inter-trial interval was 2 min. The speed of rotation was increased from 4 to 40 rpm during a 120-s period. Latency to fall was recorded automatically. The mean riding time was measured for each animal. In case of longer than 180 s riding duration, the rat was removed from the rod and the fall latency was assumed to be 180 s.

2.2.2. Open-field

This maze was used to measure the rat's exploratory and anxiety-like behavior. Animals with less anxiety, spend more time in the central area of the arena. The open-field arena was constructed using white Plexiglas with 75 cm length, 75 cm width and 36 cm high. The rats were individually placed in the center of the arena and the number of rearing, as well as the percentages of time spent in the central area of the maze was recorded during 5 min, as previously described with the time duration modification [10].

2.2.3. Y-maze

Y-maze alternation task was performed, as described above, to investigate icv-STZ effect on animals' working memory [11]. Briefly, animal was placed in the center of the maze to explore the arena freely, and spontaneous alternation behaviors were counted over an 8-min session. The function of working memory of animals was quantified as the percentage of alternation: (the number of alternations) / (total arm entries − 2) ∗ 100. Also, the number of arm entries was recorded as a locomotor activity of rat during an 8-min session of Y-maze alternation task. The apparatus consisted of three equal arms, measuring 40 × 15 × 30 cm oriented at 120° angles from each other. The number of different arm visits was considered as an index of exploratory activity of the animal in the Y-maze.

2.2.4. Novel object recognition test

This test has been designed to assess animal's recognition memory, as well as working memory, based on the rodent's preference to explore new objects. Nevertheless, animal's reference memory is not involved in this test. These advantages make this test useful to evaluate mild cognitive impairment in pre-clinical studies [12]. Here, based on rat's unconditioned preference for novelty, short-term, medium-term and the long-term effect of different doses of icv-STZ were evaluated, as reported previously with some modifications [13]. Briefly, the novel object recognition test was performed in three phases of habituation, familiarization (acquisition phase) and test (retention phase). During habituation phase, the animals were individually placed in the apparatus (an empty open-field arena measuring 75 × 75 × 36 cm) to explore the arena for five consecutive days, 15 min per day. On the test day, two identical objects (colored striped plastic bottles filled with NaCl) were placed in the opposite corners of the arena during the acquisition phase; the animal was released in the arena back to the objects and allowed to explore the objects freely for 3 min. Exploration was defined as pointing the head within 2 cm of the objects, sniffing, licking or biting the objects. Duration of time each rat explored each object was