Thai Journal of Pharmaceutical Sciences (TJPS)

5th International Conference on Pharmaceuticals,
Nutraceuticals and Cosmetic Science (IPNaCS 2017)

Comparative Behavioural Study of Unilateral and Bilateral Intrahippocampal Injection of Streptozotocin for Alzheimer's Disease Rat Model

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Keywords: Alzheimer's Disease, Rat Model, Streptozotocin, Morris Water Maze, Unilateral and Bilateral Injection

Introduction

Alzheimer's disease (AD) is a slow degenerative brain disorder that causes memory loss and other cognitive impairments. Sporadic AD (sAD) has contributed to 99% of AD cases compared to familial AD (fAD) that mostly caused by genetic, environmental, and metabolic factor. To study Alzheimer's disease, various models were reported by researchers.[1-3] Rodents are commonly used as AD model to understand the disease process and to find new therapeutic interventions. Thus, it is important that rodent model for AD mimic the disease pathology. The hallmarks of AD disease include formation of amyloid beta plaques and neurofibrillary tangles that eventually lead to the damage and death of the neurons.[4]

Existing neurotoxin-induced models of AD utilizes compounds such as streptozotocin (STZ), okadaic acid, and colchicine. STZ-mediated induction causes deterioration of memory and creates many features commonly found in human sAD.[5] To induce AD, STZ can be injected into the brain either by intracerebroventricular (ICV) or intrahippocampal (IH) route. The ICV method is frequently used by researchers[2,6-7] to develop the AD model but not the IH. The STZ is an insulin receptor (IR) disruptor which is associated with glucose hypometabolism, oxidative stress, neurodegeneration, and memory impairments that are commonly found in sAD.[5] The IR in the brain are abundant in the hippocampus specifically in the *Cornu Ammonis* 3 (CA3) region which associated with memory deficit. Though the IR is abundant in the hippocampus, the effect of injecting STZ in this region has not been elucidated. Therefore, injection of STZ in the hippocampus will possibly be another method for producing another AD non-transgenic rat model.

This research aimed to study the effects of STZ injection in the IH unilaterally and bilaterally. The behavioural abnormalities were studied using Morris Water Maze (MWM) as this assessment is considered one of the best methods to investigate cognitive and memory impairments in non-transgenic rodents' model of AD. The method evaluated the cognitive processed of neural network such as hippocampus and cortical brain regions.[8]

Methods

Animals

Four-month-old male Sprague-Dawley rats (350–450 g) were obtained from the Laboratory Animal Facility and Management (LAFAM), Universiti Teknologi MARA (UiTM), Malaysia. Animals were housed individually per cage, maintained at 23 ± 2 °C, with free access to food and water *ad libitum*. The protocols were reviewed and approved by the Institutional Research Committee on the Ethical Use of Animals (162/2017) (UiTM Care).

Injection of STZ

Streptozotocin purchased from Sigma-Aldrich (Malaysia) was dissolved in phosphate-buffered saline (PBS – 0.01 M NaH_2PO_4, 0.137 M NaCl, pH 7.4). The solution was freshly prepared prior to surgery. Animals were randomly divided into five groups. The control group rats were not subjected to any surgery and were kept in the same condition as the other groups. Two treatment groups received unilateral (10 µl) and bilateral intrahippocampal (5 µl each side) injections of STZ (3 mg/kg bw) respectively. While another two sham groups

received equal volume of PBS unilaterally and bilaterally. Prior to surgery, the animals were anesthetized intraperitoneally with ketamine (75 mg/kg bw) and xylazine (10 mg/kg bw) (Troy Ilium, Australia). The top of the skull was shaved and sterilized before positioning the animal on a stereotaxic apparatus (Stoelting, USA). The bregma and skull surface was the stereotaxic zero point. Two small holes were drilled in the parietal bone posterior to the bregma with coordinates at -3.6 mm anterioposteriolly (AP), -2.0 mm for lateralmedially (LM) and -3.4 dorsoventrally (DS) (for bilateral injection the LM coordinates was ± 2.0 mm). A 25 µl Hamilton syringe (22-gauge) was placed on the surface of the skull hole and the needle tip was slowly inserted into the injection point of the brain. Following suture of the wound, all rats received an intramuscular injection (0.25 ml) of penicillin (Gibco, Life Technology, USA) and were individually housed for recovery with food and water available *ad libitum*.

Morris Water Maze (MWM)

The maze consisted of a circular pool (150 cm in diameter and 40 cm height) filled with water to a depth of 30 cm maintained at 28 ± 1°C). The pool was equally divided into four quadrants (North, South, East and West) with an escape platform positioned at one of the center of these quadrants and submerged approximately 1.5 cm below water surface. The MWM test consists of the hidden platform task (HP) and probe trial task (PT). Coloured cues were used to help the animals remember the platform location and the pool was filled with coloured water using non-toxic black dye to hide the escape platform. The rats were allowed to swim freely in the pool for familiarization. For the HP task, the rat was placed in the maze facing the wall and gently into the water starting at one of the quadrant. The rat was allowed to swim freely for one minute and to find the platform to escape. Rats failed to escape within that time were guided to the platform and remained on it for 20 s. The HP tests was conducted for four consecutive days. The test included three trials for each day. A probe test was conducted 24 h after the end of the HP test which is at day 5. During this test, the platform was removed and the animals were allowed to swim freely in the pool for one min. The data were analysed with ANY-maze software version 4.99 and - Statistical Package for Social Sciences (SPSS®), version 23.0 (independent t-test).

Results

MWM: Acquisition of spatial memory

Figure 1 shows the effects of STZ injected in the hippocampus area unilateral (left) and bilateral (right). No difference was noted in the result between control and sham groups. Thus, we only reported the finding of sham and the STZ treated group. In general, escape latency (EL) and total distance (TD) travelled by the sham group decreased gradually over the four days of training. In the EL task, both the STZ administered unilateral and bilateral took longer time to reach the platform compared to the sham animals. The results of unilateral injection showed significant values for day 4 of MWM task in (EL) (p = 0.025)(Figure 1:A (left)) and (TD) (p = 0.027))(Figure 1:B (left)). Meanwhile, the bilateral injection of STZ showed significant values for day 1 until day 4 for both EL (Figure 1:A (right)) and TD (Figure 1:B (right)) (EL: Day 1 (p = 0.007), day 2 (p = 0.011, day 3 (p = 0.023), and day 4 (p = 0.044); TD: Day 1 (p = 0.005), day 2 (p = 0.004, day 3 (p = 0.019), and day 4 (p = 0.018)).

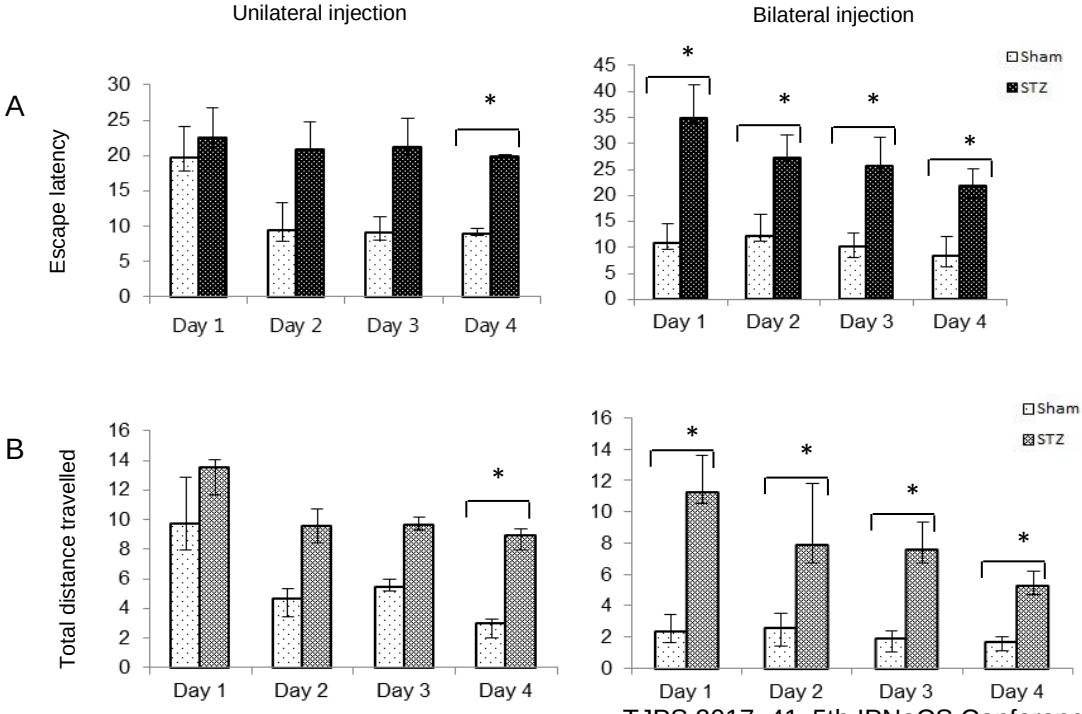

Figure 1 The effects of unilateral (left) and bilateral (right) intrahippocampal STZ administration on rats' acquisition of spatial memory: A. escape latency (s)(top) and B. total distance travelled (m)(bottom). Data are reported as mean ± SD, * $p < 0.05$ vs sham group.

MWM: Retrieval of spatial memory (Probe test)

During the PT, retrieval of spatial memory was analysed. Both groups showed shorter time spent in the target quadrant by the STZ treated rats compared to sham. However, only the bilateral group showed significant result ($p = 0.024$) (Figure 2).

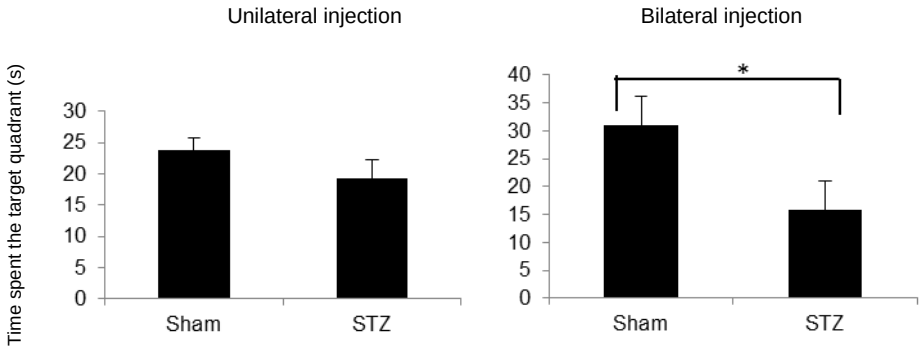

Figure 2 The effects of STZ treatment administered unilaterally (left) and bilaterally (right) on rats' performance in probe trial test. The time of the rats spend in the target quadrant. Data are reported as mean ± SD, * $p < 0.05$ sham group.

Discussion

The results of this study showed that both unilateral and bilateral injection of STZ in the hippocampus of the brain elicited impairment of memory 12 weeks after STZ treatment. The results revealed that both STZ injection methods were able to cause learning and memory deficits. However, the results were more prominent via bilateral STZ administration. Thus, we suggest that the bilateral administration of STZ produces an alternative to the existing rodent sAD model. In this case the IR in both sides of the hippocampus were destroyed compared to only one site in the unilateral administration. In this study, both unilateral and bilateral injection of STZ in the brain has low mortality rate (7%) compared to a study reported by Sharma and Gupta (2001).[9] The AD-like models are critical to evaluate the disease as they can mimic the disease pathology and can reproduce the complexity of the disease.[3] Kraska et al. (2012) reported that at high concentration (3 mg/kg of STZ) of STZ induced lesions in septum and corpus callosum that lead to neuronal loss.[10] The authors used the high-field magnetic resonance imaging (MRI) to detect cerebral abnormalities and neurodegenerative lesions formed in the septum and corpus callosum.

The EL showed decrease in time as days increase. Sagi et al. (2012) reported that a single day of MWM training can cause rapid learning changes[11] which can be associated with neuroplasticity. The hippocampus of the brain consists of CA1, CA2, CA3 and subiculum region with CA3 reported as the most negatively affected region by STZ.[12] It was proposed that the hippocampus CA3 region is responsible for spatial memory processes.[13] The result of the time spend in the target quadrant showed that the STZ treated rats were spending less time in the target quadrant compared to the sham group. This result was supported by other reports.[1]

Conclusion

This study revealed that the AD-like model was produced by injection of STZ in the hippocampus area either unilateral or bilateral at concentration of 3 mg/kg. Overall, this study pointed out that bilateral injection of STZ intrahippocampally is a more suitable as AD model that can be used to develop effective therapies[7,14-15] for AD.

Acknowledgements

The authors involved in the research would like to thank Brain Laboratory Faculty of Pharmacy, UiTM, Puncak Alam Campus for providing the facilities. This research was supported by Fundamental Research Grant Scheme (FRGS), Malaysia (600-RMI/FRGS 5/3(28/2015).

References

1. Zussy C, Brureau A, Keller E, Marchal S, Blayo C, Delair B, Ixart G, Maurice T, Givalois L. Alzheimer's disease related markers, cellular toxicity and behavioural deficits induced six weeks after oligomeric amyloid-β peptide injection in rats. Plos One. 2013;8(1):e53117.
2. Knezovic A, Osmanovic-Barilar J, Curlin M, Hof PR, Simic G, Riederer P, Salkovic-Petrisic M. Staging of cognitive deficits and neuropathological and ultrastructural changes in streptozotocin-induced rat model of Alzheimer's disease. Journal of Neural Transmission. 2015;122(4):577-592.
3. Singh A, Kumar A. Comparative analysis of intrahippocampal Amyloid Beta (1-42) and it is intracerebroventricular streptozotocin models of Alzheimer's disease: Possible behavioural, biochemical, mitochondrial, cellular and histopathological evidences. Journal of Alzheimer Disease Parkinsonism. 2016;6(1):208. Doi: 10.4172/2161-0460.1000208.
4. Karantzoulis, S, Galvin JE. Distinguishing Alzheimer's disease from other major forms of dementia. Expert Review of Neurotherapeutics. 2011;11(11):1579-1591.
5. Kamat PK. Streptozotocin induced Alzheimer's disease like changes and the underlying neural degeneration and regeneration mechanism. Neural Regeneration Research. 2015;10(7):1050–1052.
6. Liu P, Zou L-B, Wang L-H, Jiao Q, Chi T-Y, Ji X-F, Jin G, Xanthoceraside attenuates tau hyperphosphorylation and cognitive deficits in intracerebroventricular-streptozotocin injected rats. Psychopharmacology. 2014;231: 345-356.
7. Salkovic-Petrisic M, Osmanovic-Barilar J, Brückner M.K., Hoyer S, Arendt T, Riederer P. Cerebral amyloid angiopathy in streptozotocin rat model of sporadic Alzheimer's disease: a long-term follow up study. Journal of Neural Transmissio. 2011;118:765-772.
8. D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Research Reviews. 2001;36(1):60-90.
9. Sharma M, Gupta YK. Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. Life sciences. 2001;68:1021-1029.
10. Kraska A, Santin M, Dorieux O, Joseph-Mathurin N, Bourrin E, Petit F, Jan C, Chaigneau M, Hantraye P, Lestage P, Dhenain M. In vivo cross-sectional characterization of cerebral alterations induced by intracerebroventricular administration of streptozotocin. Plos One. 2012; 7: e46196.
11. Sagi Y, Tavor I, Hofstetter S, Tzur-Moryosef S, Blumenfeld-Katzir T, Assaf Y. Learning in the fast lane: new insights into neuroplasticity. Neuron. 2012;73(6):1195–1203.
12. Ramezani M, Darbandi N, Khodagholi F, Hashemi A. Myricetin protects hippocampal CA3 pyramidal neurons and improves learning and memory impairments in rats with Alzheimer′s disease. Neural Regeneration Research. 2016;11(12):1976-1980.
13. Florian C, Roullet P. Hippocampal CA3-region is crucial for acquisition and memory consolidation in Morris water maze task in mice. Behavioural Brain Research. 2004;154(2):365-374.
14. Deshmukh R, Kaundal M, Bansal V, Samardeep. Caffeic acid attenuates oxidative stress, learning and memory deficit in intra-cerebroventricular streptozotocin induced experimental dementia in rats. Biomedicine & Pharmacotherapy. 2016;81:56-62.
15. Shingo AS, Kanabayashi T, Kito S, Murase T. Intracerebroventricular administration of an insulin analogue recovers STZ-induced cognitive decline in rats. Behavioural Brain Research. 2013;241:105-111.