Streptozotocin induces cognitive impairment in rats*

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Alzheimer's disease (AD) is a progressive neurodegenerative disease, and one of the most common causes of dementia. It is clinically characterized by irreversible and progressive decline in cognitive functions. The effective therapeutic drugs in the clinic are still lacking. Ideally, AD progression could be stopped at an early stage. This study was designed to investigate the potential effect of Streptozotocin in male Wister rats on cognitive function. Animal was injected with single bilateral intraceroventricular injection of STZ (3 mg /kg). Cognitive function assessment was assessed one month later with Morris Water Maze and Y Maze tasks. **Results** Cognitive impairment caused by ICV-STZ was evident in MWM by elongation in Escape latency time and decrease time spent in target quadrant and decrease in spontaneous alteration ratio in Y Maze. **Conclusion** Our study indicated that STZ impaired neural plasticity induced neuronal loss in both the cortex and the hippocampus, and a state of dementia was induced, feasibly indicating a state of AD.

1- Introduction

Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder. It is characterized by memory loss and cognition impairment accompanied by personality changes and abnormal behavior. AD is the most common cause of dementia as it accounts as 60-70% of dementia cases according to the world health organization (WHO) [(1)].The change in world age demography towards old age increases the neurodegenerative diseases risk including AD [(2)]. AD is considered an important public health problem due to its high prevalence and heavy socioeconomic costs for society [(3)].AD consists mainly of two types Familial type

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accounts 1-5% of AD cases and Sporadic type presents 95% of cases [1]. Many factors contribute in AD pathology development. Genetics, environment, toxic substances exposure, age, depression, neurotrophic factors deficiency, brain trauma are among these factors [4].

The key neuropathological features of AD include deposition of extracellular amyloid plaque and intracellular neurofibrillary tangles (NFT) starting in hippocampus then stretching out to cortical grey matter[5] Amyloid beta (Aβ) plays a crucial pathogenic role in disease development, both in familial and sporadic types [6]. β amyloid deposition leads to synaptic degeneration , tau-hyper phosphorylation, neuroinflammation, oxidative stress and neuronal cell death[7].

The balance between Phosphorylated and unphosphorylated tau keeps the steadiness of microtubule structure Excessive tau phosphorylation at (up to 21 epitopes) leads to instability of microtubules and cell death this what happens in AD [6]. Several factors and mechanisms are involved in AD pathogeneses. Among these factors a well-established mechanism found in sporadic type of AD is insulin signaling dysfunction (insulin resistant brain state) [8]. Insulin and insulin signaling molecules play important roles in synapse physiology and plasticity supporting their role in learning an d memory [9]. To date despite intense research the currently available treatments are only symptomatic improve patient quality of life without disease-modifying effects. So, there is an urgent need to continue research and to test various treatments and compounds and

explore different therapeutic strategies for AD and bring treatment as early as possible to slow the fast and progressive nature of the disease.

Streptozotocin (STZ) [2-deoxy-2-(3-(methyl-3-nitrosoureido)-D glucopyranose) derived from Streptomycetes achromogenes soil bacteria initially created as anticancer agent and widely used as a diabetogenic compound on experimental animals has been found that single or double intracerebroventricular (ICV) STZ injection(s) diminish cerebral glucose uptake and produce multiple effects that mimics AD features especially sporadic type at the pathological ,molecular, neurochemical and behavioral levels[10].

STZ is used in a subdiabetogenic dose (3 mg/kg) to develop AD pathology. It has structural similarity to glucose and cellular uptake of STZ occurs through Glucose transporter 2 (GLUT2) [9]. when injected systemically ,it can't affect brain directly as blood brain barrier lakes GLUT2, so it is injected intracerebroventricular (ICV) to develop AD model and ICV administration of STZ dosen't alter blood glucose level [11].

ICV administration of STZ leads to increase Aβ and tau protein expression in cerebral cortex and hippocampus after 3 weeks of treatment reducing spatial memory recognition and increasing mortality. Aβ and tau proteins are the starting point of the pathological cascade that occurs in AD resulting in dementia, neurobehavioral changes and eventually neural death. In addition it causes oxidative stress, astrocyte and microglial activation releasing

inflammatory mediators and biochemical alteration [9], cholinergic deficit [10].

2- Materials and methods

2.1. Experimental animals: All animal procedures were approved by the Institutional Animal Ethics Committee for Ain Shams University, Faculty of Medicine. Male Wister rats weighing about 250-300 g were kept in a standard laboratory conditions. Rats were housed in a quiet, temperature and humidity controlled room with 12:12 h light/dark cycle and ad libitum access to food and water. Animals were acclimatized to laboratory conditions at least for one week before the tests.

2.2. Drugs &chemicals: Streptozotocin (STZ) *(MP Biomedicals, LLc , France)* dissolved in citrate buffer Ketamine hydrochloride (*TRITTAU, Germany)* Xylazine Hydrochloride *(ADWIA co, Egypt)*. All solutions were fresh prepared before administration.

2.3. Experimental procedures

2.3.1. Intracerebroventricular administration of streptozotocin:[12] Rats were weighted and marked at the base of their tail . Rats were anesthetized with ketamine hydrochloride (70 mg/kg) and Xylazine (9 mg/kg) .Operating table was disinfected by 70% alcohol and all used surgical instruments were sterilized. The head was fixed in Sterotaxic frame and the operative site was shaved, cleaned 70% alcohol swab twice. Midline sagittal incision was done on the scalp, the periosteum was resected and the skull land marks was exposed and a hole was drilled according to the following co-ordinates 0.8 mm posterior to bregma , 1.5 mm lateral to sagittal suture and 3.6 mm ventral from the surface of the brain according to rat atlas. STZ was fresh prepared by dissolving in cold citrate buffer (pH 4.4) just prior to administration. STZ group was injected ICV with STZ bilaterally at a dose of 3 mg/kg, only once on day one. The concentration of STZ in citrate buffer was adjusted so as to deliver 2 µl of the solution on each

side with a flow rate 0.5µl/minute using 5 microliter Hamilton syringe. Sham group received the same amount of citrate buffer ICV bilaterally once on day one as STZ injected rats.The cut skin was sutured and 1 ml saline was administrated subcutaneous to prevent dehydration followed by analgesic and systemic antibiotic for three days postoperative in heated cages with topical antiseptic spray. The body weights were monitored throughout the study period.

2.3.2. Study design: Rats were randomly assigned into 2 groups (n=10) after a week of acclimatization. Group I: rats were injected with Citrate buffer Intracerebroventricular (ICV) and received saline with oral gavage for one month . Group II: rats were injected with Streptozotocin (STZ) (3mg/kg, ICV) and received saline with oral gavage for one month.

2.3. 3 Parameters measured

At the end of the experiment these parameters were measured

3- Behavioral parameters:

Morris Water Maze

Water Maze (13) consists of a large circular plastic tank (180cm in diameter, 80 cm height) filled with water up to 40 cm below the edge maintained at about 25± 2°C which made opaque using white powdered nontoxic material. Submerged platform 2 cm below water surface (10 cm diameter, 38 cm height) non visible to the swimming rats. The entire set up was performed in a quiet room. Stationary geometric cues made by different objects placed on the four cardinal points of the maze and could be used by the rats for spatial orientation. The examiner represents a distal cue for swimming rats so the cues and the examiner remained in a constant location throughout the study. The Maze is divided into 4 quadrants by a cross. Submerged platform was present in quadrant

4 (The target quadrant TQ). The Maze test was carried out as follow acquisition sessions four trails per day for four consecutive days starting from 16 to 19 day of the study.

A trial started as soon as the rats were placed in the pool, facing towards the wall of pool. The trial terminated as soon as the rat found the platform or when 90 s had elapsed. The rats were allowed to stay on platform for 5 s. The rats were taken out and a fresh trial was started. Any rat did not find the platform within 90 s, the trail was considered over and it was gently guided to the platform and allowed to stay there for 10 s. on the fifth day, a probe trail was performed with the same protocol except that the platform was removed and the tested rat was allowed to swim freely once for 6o s to find the platform. Escape latency time in seconds (the time to reach the target quadrant) and the time spent in the target quadrant in a total 60 s were measured.

4- Y Maze

Y-maze test [14] used to measure the spatial working memory in rats.. Our apparatus made of transparent acryl and it consisted of three arms (A, B, C) at 120° of each other making the Y shaped maze. Each arm was measured (40, 30, 15) cm length, height and width respectively. Rats were put at the end of one arm and left to explore the maze for 8 minutes .Rats tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the rats know which arm they have already visited. Arm entry sessions were recorded as follow (ACBCBAACBA) a valid arm entry was recorded when the hind paws of the rats were completely placed in the arm. Consecutive entry into three arms in an alternative order was defined as successive entries. Spontaneous

alternation was calculated according to following formula: [(number of alternations)/ (total number of arm entries − 2)] × 100 .The cognitive function of rats is measured
by the spontaneous alteration ratio.

Statistical analysis: The statistical analysis carried out on the software program, Graph pad prism version 4 (2005), USA. Data were presented as mean ± SD. statistical difference among groups was determined using Unpaired T test for comparison between two groups a p value less than 0.05 were considered statistically significant.

5- Results

3.1. Morris Water Maze (MWM).

Table 1: Demonstrate effect of STZ after one month in Morris Water Maze performance. Escape latency time during acquisition phase four days and escape latency time in the fifth day during probe trail. Data presented as mean ± SD with significant difference ($p<0.05$)

Results revealed that during the acquisition phase. The escape latency time was significantly ($p<0.05$) longer in STZ untreated group compared to control.

Day	Escape latency time (S) n (10)	
	Control	STZ group
1	54.77 ± 7.46	82.15±5.92*
2	34.09±9.58	76.59±9.16*
3	21.13± 6.88	60.87±14.54*
4	14. 72± 4.84	54.68±16.43*
5	5.66±1.22	34.21±8.21*

*significant difference $p < 0.05$ compared to control
n number of animals.

Table 2: Effect of STZ for one month on learning and memory in Morris Water Maze test (Probe Trial). Demonstrate percent of time spent in target quadrant in a total 60 s among different groups.

During probe trail , STZ untreated group took longer time to reach the target quadrant and spend much less time in target quadrant in a total 60 S compared to control.

Group n=(10)	Time spent in target quadrant in a total 60 s	
	Control	STZ
Rat 1	35.0	25.0
Rat 2	41.6	21.6
Rat 3	31.6	18.3
Rat 4	50.0	13.3
Rat 5	60.0	18.3
Rat 6	75.0	13.3
Rat 7	35.0	10.0
Rat 8	40.0	10.0
Rat 9	50.0	1.0
Rat 10	45.0	15

n number of animal per group
$p < 0.0001$ unpaired t- test

3.2. Y Maze

Table (3): Demonstrate effect of STZ after one month on spatial memory deficit in Y Maze test in STZ induced AD rat model

Results revealed that Streptozotocin had significantly ($p < 0.05$) induced reduction in spontaneous alteration performance ratio compared to control group

Group (n=10)	Spontaneous alteration performance %	
	Control	STZ
Rat 1	85.71	25.00
Rat 2	60.00	33.33
Rat 3	66.66	40.00
Rat 4	60.00	80.00
Rat 5	90.00	16.66
Rat 6	70.00	33.33
Rat 7	85.71	35.29
Rat 8	60.00	15.00
Rat 9	70.00	20.00
Rat 10	75.00	17.00

n number of animal per group
$p < 0.0001$ unpaired t- test

4. Discussion

This study was designed to investigate the potential effect of Streptozotocin in male Wister rats on cognitive function. Intracerebro-ventricular(ICV) administration of streptozotocin (STZ) produce insulin resistant brain state and induce behavioral, chemical and histological changes resembling human AD [15]. In the present study, bilateral ICV administration of STZ showed deterioration of learning and memory abilities in animals. There was

significant difference in cognitive behavioral tests performance among control and STZ group in accordance with previous studies (12) [16]. Morris water Maze test and Y Maze were employed for learning and memory evaluation. STZ induced poor performance in MWM test by decreasing escape latency time and increasing time spend in target quadrant which was significant in comparison to control group showing poor learning and indicator for impairment in spatial memory. To confirm MWM results, we tested spatial cognition and memory with Y maze test. STZ decreases spontaneous alteration performance in Y Maze test with significant difference to control group.

Conclusion, from the above results we can conclude that Intracerebroventricular administration of Streptozotocin caused cognitive dysfunction and considered a suitable model for exploration the effect of various agents on cognitive

5. References

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الستربتوزيتوسين وما يسببه من ضعف للوظيفة المعرفيه فى الجرذان

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مرض الزهايمر هو اضطراب عصبى تقدمى ساحق يتميز بفقدان تدريجى للذاكرة وللوظيفة المعرفية. وهو يمثل السبب الرئيسى للخرف فى جميع أنحاء العالم ولا يوجد له علاج فعال حتى وقتنا الحالى مع وجود احتمالية لوقف تقدمه فى مرحلة مبكرة، صممت هذه الدراسة لاستقصاء التأثير المحتمل للستربتوزوتوسين فى جرذان ويستر الذكور على الوظيفة المعرفيه، حيث تم حقنهم بالستربتوزيتوسين ٣ مغ / كج داخل بطين الدماغ الأيمن والأيسر مرة واحده فى اليوم الأول من الدراسة. تم تقييم الوظيفة المعرفيه بعد شهر واحد من خلال مهام متاهة موريس ومتاهة ال Y كان الضعف المعرفى الناجم عن الستربتوزيتوسين واضحا فى متاهة موريس من خلال الاستطالة فى وقت زمن الوصول للهروب وتقليل الوقت الذى يقضيه فى الربع المستهدف وانخفاض فى معدل التغيير التلقائى فى متاهة الـ Y.

استنتجت دراستنا أن الستربتوزيتوسين قد أضعف اللدونة العصبية التى تسببت فى فقدان الخلايا العصبية فى كل من القشرة والحصين، وتم إحداث حالة من الخرف مشيرًا لمرض الزهايمر.