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ORIGINAL ARTICLE

Effects of Duration of Diabetes on Cognitive Functions in Streptozotocin Induced Young Diabetic Rats

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Abstract: *Background:* Children with early onset Type I diabetes have been reported to show modest deficits on a wide range of neuropsychological tests. However, the contribution of duration of the disease with respect to cognitive dysfunction remains unresolved. The present study aims to determine the effects of different duration of hyperglycemia on cognitive function in young diabetic rats. *Objectives:* To determine the effect of 10 and 20 days duration of diabetes on cognitive functions. *Materials and methods:* Diabetes was induced in young rat pups, by streptozotocin injection (i.p) at a dose of 50 mg /kg.BW. Diabetic state was confirmed on 30th post natal day. 10 and 20 days after inducing diabetes, the rats were tested in passive avoidance box and Morris water maze, over a period of 10 days. *Results:* Diabetic rats showed significantly impaired cognitive functions, compared to their age matched controls. The cognitive impairment was greater in rats with 20 days of diabetic state compared to their 10 days counterparts. *Conclusion:* It is essential to diagnose and treat diabetes as early as possible in case of young children with Type I diabetes of early onset to prevent irreversible cognitive functions.

Key words: diabetes, cognition, learning, Morris water maze, passive avoidance

Introduction

Long-standing concern about the effects of Type I diabetes (T1DM) on cognitive ability has enhanced with the increasing incidence of early onset T1DM in children. A recent meta-analysis clearly shows that cognitive function is mildly impaired in patients with T1DM, which is mainly reflected in a slowing of mental speed and a diminished mental flexibility [1]. Lowered cognitive performance in diabetic patients appeared to be associated with several factors. Both chronic hyperglycemia [2-3] and the consequent occurrence of diabetes complications [4], as well as recurrent episodes of severe hypoglycemia [5], are thought to be associated with cognitive dysfunction in patients with type 1 diabetes. However, the combined effects of juvenile onset and the occurrence of diabetes complications may have an impact on cognition [6-7]. Many studies support the report that a high level of HbA_{1c} in interaction with diabetes complications such as neuropathy might negatively influence cognition [8-14]. Although the magnitude of most of these cognitive decrements is relatively modest, even moderate forms of cognitive dysfunction can potentially hamper day to day activities. These may present problems in more demanding situations and can have a negative impact on the quality of life.

Studies that assessed cognition in children with diabetes observed that a very early age at onset (before the age of 5 years) does appear to be associated with more severe impairments of cognitive performance [15-16]. In particular, patients with diabetes onset before the age of 5 years could be more sensitive to the effects of hypoglycemic episodes or elevated HbA_{1c} levels. Thus many researchers have shown that there is a relationship between cognitive dysfunction and type 1diabetes of early onset [17-18]. The questions that still remained unresolved are contribution of different disease variables, such as diabetes duration, levels of glycemic control and the development of micro vascular complications to cognitive impairment. Hence, the present study was under taken to evaluate the effects of different duration of diabetes state on various learning and memory as well as behavioural parameters in diabetic young rat pups.

Material and Methods

Animals: Inbred, Wistar strain albino male and female, 25 days old rats were selected for this study. Experiments were carried out after obtaining Institutional Animal Ethical Committee approval *(627/02/a/CPCSEA dated 17/7/2008).* The animals were maintained under 12:12 hours dark: light cycle and controlled temperature (25± 3°C). Animals were fed with food (Amruth feeds, standard rat pellets) and water *ad libitum.* All the experiments were performed between 08:00-16:00 hrs.

Experimental design: Animals were divided into two groups, each with two subgroups (n = 6). Two subgroups served as normal and diabetic control for 10 days group, whereas another two subgroups for 20 days normal and diabetic group. Rats in diabetic groups received Streptozotocin injection at a dose of 50 mg/kg BW intraperitonially, on 25th day of postnatal life. After four days, blood samples were collected from tail vein following overnight fasting [19-21]. Fasting blood sugar (FBS) was estimated with the help of a standard glucometer (Optium, Germany) and the day of confirmation of diabetes was considered as day one of diabetic state. Those animals with FBS levels higher than 200 mg% were included in the study. From day 11 to day 20, after diabetes induction, rats in 10 days groups were tested for various cognitive and behavioral parameters, in elevated plus maze, two compartment test for passive avoidance and Morris water maze. Similarly, rats in 20 days groups were also studied for the same parameters, from day 21 to day 30 after diabetes state was confirmed. The details of each test are as follows:

Morris water maze (MWM): The spatial learning abilities and memory of rats were assessed in the MWM task. The water maze consists of a circular pool (diameter, 1.5 m; height, 60 cm), filled with water (26°C±1^{0}C) in which a square (5 cm) escape platform was hidden 1 cm below the surface of the 30 cm. deep water. Water was made opaque by adding milk. The water maze was divided into four equal imaginary quadrants and escape platform was kept in a constant place, the center of the north-east quadrant. The maze was located in an experimental room containing relevant visual cues. Rats were trained for three consecutive days, daily sessions consisting of 3 trials with 5 min. of inter trial interval. In each trial, rats were given 120 sec. to swim and find the escape platform.

In case rats failed to find the platform within 120 sec, they were hand guided onto the platform. All animals were allowed to rest on the platform for 30 sec. A farthest starting location from platform was used in each trial. The time taken by each rat to reach the hidden platform was measured (transfer/escape latency). On 4th day, probe trial was conducted during which the platform was removed from the maze and the rats were allowed to swim freely in the pool. The time spent in the target quadrant which has the platform in the previous trials, was served as measure of memory [22-23].

Passive avoidance test: The two compartment passive avoidance apparatus was used to assess the associative learning and memory. Essentially the apparatus consists of a square box with a floor grid of 50 x 50 cm and wooden walls of 35 cm height. A 100 watts bulb illuminates this box. In the center of one of the walls is an opening of 6 × 6 cm which can be opened or closed using a transparent glass sliding door. This opening leads to a smaller (15 × 15 X 15 cm), dark compartment provided with an electrified grid floor that can be connected to a shock source (stimulator). Animals were placed individually in an illuminated chamber facing away and at the farthest distance from the entrance to the dark compartment. On the 1st day of the test, each rat was allowed to explore both the compartments for 5 min. On the 2nd day, time taken to enter the dark compartment for the 1st time was noted and soon two learning sessions were followed. At the end of the 3rd trial, as soon as the rat entered the dark compartment, the door was closed and three inescapable foot shocks (50V, 50 Hz, 1sec) were given. Then the animal was returned to its home cage. 24 hours later each animal was placed again in the passive avoidance apparatus as before for a maximum period of 5 min. The latency time required for the animal to enter the dark compartment was measured. Increased latency/absence of entry into the dark compartment indicated positive memory retention [24-25].

Statistical analysis: The results were expressed as mean ± SD. The comparison of FBS and body weight of rats was carried out by Student's unpaired 'T' test. For all neurocognitive tests, between groups' comparisons were analyzed by Mann-Whitney 'U' non-parametric test. Differences were considered to be significant at probability value (P) < 0.05.

Results

Rats, which were able to achieve the required diabetic state (FBS more than 200 mg %) after STZ injection were only included in the study. Four animals in the diabetic group died during the experiment due to severe hyperglycemia. Fasting blood sugar and body weight of rats in different groups are shown in Table 1. The statistically significant difference (P <0.001) was observed in FBS values between the rats in diabetic group, and their age matched normal control group. However, body weight of rats in both groups did not differ significantly initially. The diabetic state was worsened over the time, showing the significant difference in FBS values on 30th day and 60th day (P = 0.018) among diabetic rats in 20 days duration of hyperglycemia. Body weight was also significantly decreased with the increasing duration of diabetes in 20 days diabetic group (P <0.001).

Table-1: Effect of different duration of hyperglycemia on fasting blood sugar (FBS) levels and body weight (BW) in control and STZ-induced diabetic rats (*n* = 6 in each group) at regular interval

Group	Fasting blood sugar (mg %)			Body weight (gm.)		
	30th postnatal day	45th postnatal day	60th postnatal day	30th postnatal day	45th postnatal day	60th postnatal day
Normal control -10 days**	87.33 ± 3.85	94.6 ± 3.67	--	46.57 ± 4.00	117.5 ± 9.64	--
Diabetic control -10 days**	233 ± 10.27*	271.6 ± 15.31*#	--	46 ± 5.04	101 ± 4.12	--
Normal control-20 days	86.8 ± 3.37	92.3 ± 4.65	89 ± 3.95	50.1± 3.32	127.6 ± 13.82	128.3 ± 2.33
Diabetic control -20 days	269.1 ± 20.41*	276.6 ± 18.99*	319.33 ± 38.51*#	46.5 ± 3.36	103.5 ± 4.99	94.83 ± 8.75*$

Values are expressed as mean ± S.D;
*-Significantly different from normal counterparts ($P < 0.05$);
#-Significantly different from FBS on 30th day ($P = 0.003$) in the same group.
$ Significantly different from body weight on 30th day ($P < 0.0001$) in the same group.
** Animals were sacrificed on 50th post natal day for further tissue processing.

Table-2: Effect of different duration of hyperglycemia on memory retention in Morris water maze (*n* = 6 in each group)

Group	Time taken to reach target platform (sec)			Probe test— Time spent in target quadrant (sec)
	Day 1	Day 2	Day 3	
Normal control -10 days	65.1 ± 8.28	21.4 ± 3.45	13.3 ± 2.39	51.5 ± 7.57
Diabetic control -10 days	73.9 ± 11.05	28.9 ± 4.98	14.78 ± 3.11	28.1± 4.16*
Normal control -20 days	65.3 ± 4.96	19.9 ± 6.82	12.9 ± 3.74	57.5± 14.22
Diabetic control -20 days	133.4 ± 16.11*#	32.2 ± 7.28	15.1 ± 2.15	17.52 ± 4.97*#

Values are expressed as mean ± S.D;
* Significantly different from normal counterparts ($P < 0.05$);
# Significantly different from animals in 10 days diabetic control rats ($P < 0.05$).

The performance of experimental animals in the hidden platform test of MWM, in each group, is shown in Table 2.

During the probe test, the diabetic rats spent lesser time in the target quadrant, when compared to that of normal rats. The statistically significant difference was observed among 10 days diabetic and normal group (P = 0.022) and also in 20 days diabetic and normal controls (P = 0.021). Animals in 20 days diabetic group showed significant difference (P = 0.003) in the performance compared to animals in 10 days diabetic groups, suggesting the memory deficits worsening with duration of diabetes. Further more, during the trial sessions over three days, animals in diabetic group took more time to find the hidden platform compared to their normal counterparts. In the passive avoidance box (Table 3), 24 hours after the aversive stimuli, retention of memory was significantly different in 10 days diabetic and normal group (P <0.001) and also in 20 days diabetic rats compared to controls (P=0.004), which showed the reduced transfer latency. However no significant difference was observed in the transfer latency among the rats with 10 and 20 days duration of hyperglycemic state (P=0.244).

Table 3: Effect of different duration of hyperglycemia on memory retention in passive avoidance (n = 6 in each group)

Group	Time taken to enter small compartment (sec)				
	Day 1	Day 2			Day 3
	Trial 1	Trial 2	Trial 3	Trial 4	Transfer latency
Normal control - 10 days	88.8 ± 41.68	28.5 ± 7.58	11.6 ± 5.76	6.5 ± 0.71	206.3 ± 24.1
Diabetic control - 10 days	112.6 ± 21.87	12.8 ± 3.76	13.1 ± 4.29	9.5 ± 3.03	14.5 ± 1.61*
Normal control - 20 days	89.6 ± 43.21	9.3 ± 1.61	5.6 ± 0.33	6.3 ± 2.29	210.1 ± 30.82
Diabetic control - 20 days	150.3 ± 48.03	108.6 ± 46.72*#	61.3 ± 47.93*	55.1 ± 48.97	12.6 ± 3.40*

Values are expressed as mean ± S.D;
* Significantly different from normal counterparts (P < 0.05);
# Significantly different from animals in 10 days diabetic control rats (P< 0.05).

Discussion

In the present study, the rats were tested for increased vulnerability of cognitive dysfunction with the increasing duration of diabetic state was evaluated in young diabetic rats. Treating rats with STZ is a well-established animal model for diabetes. Streptozotocin (STZ), a β-cytotoxic, induces 'chemical diabetes' in a wide variety of animal species including rat by selectively damaging the insulin-secreting β-cells of the pancreas. Intra-peritoneal injection of STZ produces fragmentation of DNA of β-cells of pancreas which stimulates poly (ADP-ribose) and depletes NAD ultimately leading to destruction of β-cells and it is evidenced by clinical symptoms of hyperglycemia and hypoinsulinaemia [26].

MWM and passive avoidance tests were used to assess cognitive function in animals. The MWM is a well established and widely used spatial memory test for rats and mice, in which functional hippocampal integrity is essential for normal performance. Also, the passive avoidance test has been shown to be sensitive to detect deficits in associative learning and memory of rats [27]. Morris water maze, showed the evidence of impaired spatial learning in diabetic animals, compared with that of normal rats. This effect has worsened with increasing duration of hyperglycemia. The rats with 20 days of diabetes (approximately equivalent to 2 years of human life) revealed more cognitive deficits compared to their 10 days counterparts. Many studies link this cognitive deficits in diabetes to the probable mechanisms such as hyperglycemia induced end organ neuronal damage, dyslipidemia, amyloidopathy, taupathy etc [28-29]. In the present study, diabetic rats have not received any interventions like insulin, which helps to prevent neuronal damage. Hence untreated hyperglycemia for a longer duration could be one of the causes for diabetic encephalopathy. Other consequences of such insulin deficits and perturbations are innate inflammatory responses affecting synaptogenesis, neuronal degeneration. Eventually, this cascade of events leads to more profound deficits in behavioral and cognitive function due to extensive neuronal loss and decreased densities of white matter in myelinated cells. Neuroimaging data suggests the white matter atrophy in the frontal and temporal regions, which could be linked to deficits in certain cognitive domains, such as memory, information processing speed, executive function, attention and motor speed. Morphological studies of children with onset of diabetes before the age of six have revealed a high incidence of mesial temporal lobe sclerosis, which was not associated with a history of hypoglycemia [30]. Interestingly, deficits in such cognitive functions are also associated with impaired functional connectivity, a measure of functional interactions between brain regions [31]. Field excitatory postsynaptic potentials (f EPSP) recorded from hippocampal slices of diabetic animals, show defects in hippocampal synaptic plasticity induction linking to difficulties in learning and memory [32].

This study was designed to investigate the effect of different duration of diabetes on cognitive dysfunction in early span of life. Using STZ induced diabetic models; the results indicate that the duration of diabetes in younger age group has significant contribution in learning and memory deficits, which were irreversible.

Conclusion

Very recently, it has been clear that CNS is not spared by diabetes. Diabetic encephalopathy primarily caused by direct metabolic perturbations due to hyperglycemia, insulin deficiency, or hypoinsulinaemia. Secondary diabetic encephalopathy occurs as a result of micro and macro-vascular disorders, or due to repeated episodes of hypoglycemia caused by excess insulin (33-35). The results of this study suggest that learning ability and memory shows a direct relation to the duration of the diabetes; however the underlying mechanisms are yet to be established. The clinical implication of the study highlights the importance of early diagnosis and treatment of juvenile diabetes induced cognitive deficits among children.

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