

Metformin Attenuates Cognitive Deficits in Experimentally Induced Alzheimer's Disease

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Abstract: A study was undertaken to evaluate the effect of metformin on learning memory in experimentally induced Alzheimer's disease in Wistar rats. A total of 30 Wistar rats were divided in to five groups of six rats each. Group 1 served as control. In Group 2,3 and 4 Alzheimer's disease was induced by administering aluminum chloride (17 mg/kg) orally to the animals for a period of 4 weeks once daily. Group 1 and 2 received distilled water, group 3 received rivastigmine (0.3mg/kg) and group 4 and five received metformin 100mg/kg. All the rats were subjected to passive avoidance test. At the end of the experiment the rats were sacrificed and brain acetylcholinesterase level was estimated. Administration of aluminium chloride resulted in poor learning and memory and this was significantly reversed by rivastigmine and metformin. In conclusion, the memory impairment induced by aluminum chloride was reversed significantly by both rivastigmine and metformin.

Keywords: *Alzheimer's disease, Metformin, Rivastigmine, Passive avoidance test, Acetylcholinesterase.*

Introduction

Alzheimer's disease (AD) is progressive neurodegenerative disease. The pathological hallmarks of AD is associated with presence of amyloid senile plaques, neurofibrillary tangles and neuronal loss (1-4). AD is a major threat to the quality of life among elderly. Losing the independence of life and one's own personality becomes a burden to the families and caregivers. Initially, the short term memory starts declining (5). This is mainly due to neuronal dysfunction and degeneration in amygdala and hippocampus which leads to synaptic dysfunction and loss, neuronal death and eventually causes cognitive decline (1).

Diabetes mellitus (DM) affects 150 billion people worldwide. There are reports that AD is associated with brain insulin resistance, insulin deficiency and deficits in insulin signaling making DM as one of the risk factor for AD (6,7,8,). Various oral antidiabetic medications are being used in patients to maintain the blood glucose level.

Metformin is an excellent blood glucose lowering agent. Metformin on long term use has induced neurogenesis, reduced tau phosphorylation and improved spatial memory (9). Treatment of AD is currently aimed at the cholinergic and glutaminergic hypothesis. Rivastigmine, an anticholinesterase agent has shown to reduce the symptoms of memory and cognitive impairment in patients with AD (10). However the he outcome of treatment is far from satisfactory. Hence, there has been a continuous search for drugs that will control symptoms; slow, reduce and/or reverse mental and behavioral symptoms; and prevent or halt the disease. This study was aimed at evaluating the effects of metformin on memory and cognition in an experimental animal model of Alzheimer's disease (11, 12, 13).

Materials and Methods

Animals

The present study was carried out on 30 adult Wistar rats (weighing 140-200 g)

obtained from Central animal house, Manipal University, Manipal, India. The animals were maintained on normal diet and water *ad libitum*. They were housed individually and a temperature of $28 \pm 1^\circ\text{C}$, humidity $50 \pm 5^\circ\text{C}$ was maintained. 12/12-h light and dark cycle was maintained. The study was done after obtaining the clearance from the institutional ethical committee and experiments were in accordance with the guideline stated by the Ministry of Social Justice & Empowerment, Govt. of India & Committee for the Purpose of Control and Supervision on Experiments on animals (CPCSEA).

Drugs & Reagents

Drugs, chemicals and reagents were obtained commercially.

Induction of Alzheimer's Disease in Animals

Aluminium chloride (17 mg/kg) was administered orally to the animals for a period of 4 weeks once daily to induce Alzheimer's disease. (12, 14)

Adult Wistar rats were randomly allocated into five groups of six animals each. Group 1 : normal healthy animals serving as the control group; Group 2: Aluminium chloride induced disease model; Group 3 : Aluminium chloride induced AD treated with oral rivastigmine (0.3 mg/kg body weight) ; Group 4: Aluminium chloride induced AD treated with i.p. metformin (100 mg/kg body weight) ; Group 5 : normal healthy rats treated with i.p. metformin (100 mg/kg body weight).

Passive Avoidance Test

Passive avoidance test assesses learning, memory and fear-motivated avoidance task. The test is carried out in three phases: 1) exploration test, 2) an electrical stimulation and learning test and 3) memory retention test. The apparatus has two parts- one well lighted big compartment-and one small dark compartment. The light compartment is in a form of a square box – 3 walls made up of wood and one wall made of plexiglass.

The base of the dark compartment is made up of stainless steel grid. Light and dark compartment are separated by a sliding door.

During the procedure the light compartment was lighted by a 15 watt bulb. The experiment was started with exploration trial. During this trial the sliding door between the two compartments was kept open. The animal was placed away from the dark compartment gate and allowed to explore the apparatus three successive times for three minutes each. In each trial, the total time taken by the animal to enter the dark compartment was noted using a stop-watch. At the end of the trial, the rat was replaced back in the home cage, where it remained during an inter-trial interval of 5 minutes.

After the last exploration trial, the rat was again placed in the light compartment. When the animal entered the dark compartment, the sliding door between the two compartments was closed and three strong foot shocks (50 Hz, 1.5 mA, and 1 s duration) were delivered at 5-second intervals. The ceiling was then opened and the rat was replaced back in its home cage.

Short term memory retention test was carried out one hour after the trials were over. The latency time required for the animal to enter the dark compartment was recorded. The latency time was recorded as 2 minutes for those animals that did not enter the dark compartment within 2 minutes was carried out after 1 hour of receiving the aversive stimuli. Total time spent by the rats in each of the dark and light compartments during the memory retention trial was recorded. Absence of entry into the dark compartment and more time spent in the light compartment indicated positive memory retention. (15)

Brain Tissue Sampling and Preparation

At the end of the experiment, animals were sacrificed by cervical decapitation. The whole brain tissue was carefully dissected out and thoroughly washed with ice-cold phosphate buffer. Hippocampus was dissected out and homogenized in phosphate buffer (10% w/v, pH 7.4). The homogenate was centrifuged at 3000 rpm for 10 minutes at 4°C . The supernatant was utilized for estimating the levels of AChE.

Anticholinesterase (AChE) Assay

AChE activity was determined using the colorimetric assay of Ellman et al. with necessary modifications. The cloudy supernatant obtained as described above was used for this assay. This assay was performed by Acetylcholine iodide as the final substrate. Enzyme activity was carried out spectrophotometrically at 420 nm (12).

Statistical analysis: Results were expressed as mean $\pm$ SE of the mean. Data was analysed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test in the SPSS software, version 20. The level of significance was set at $p < 0.05$.

Results

Table 1: Effect of drug treatment on passive avoidance group

Groups	Latency to enter dark compartment in retention trial(seconds)	Total time spent in dark compartment in retention trial (seconds)
Control	101.2 $\pm$ 11.6*	9.6 $\pm$ 7.3*
Disease model	27.2 $\pm$ 10.7	56.6 $\pm$ 12.9
Disease model treated with rivastigmine	108.0 $\pm$ 8.0*	1.2 $\pm$ 0.80*
Disease model treated with metformin	117.0 $\pm$ 3.0*	1.0 $\pm$ 0.6*
Normal rats treated with metformin	72.0 $\pm$ 25.5	31.6 $\pm$ 15.6

All data are expressed as mean $\pm$ SEM.

Disease model- Aluminium chloride treated

p value < 0.05 vs. disease model; n=6

Acetylcholinesterase Activity

The results in table 2 showed that Aluminium chloride treated group showed an increased level of acetylcholinesterase indicating decline in memory. Rivastigmine

Passive Avoidance Test

The result in table 1 shows that Aluminium chloride treated group significantly decreased the step-through latency compared to control group ($p < 0.05$). During the retention trial, Aluminium chloride treated group spent more time in the dark compartment compared to the control group, indicating memory impairment whereas rivastigmine and metformin treated group showed a significant increase in step-through latency compared to Aluminium chloride treated group ($p < 0.05$), indicating reversal of memory impairment.

and metformin treated group showed a decrease in acetylcholinesterase level compared to Aluminium chloride treated group indicating a reversal of memory impairment.

Table 2: Effect of drug treatment on Acetylcholinesterase activity

Groups	Acetylcholinesterase activity(μ mol/mg/min)
Control	0.73 $\pm$ 0.16*
Disease model	1.40 $\pm$ 0.15
Disease model treated with rivastigmine	0.91 $\pm$ 0.02*
Disease model treated with metformin	0.92 $\pm$ 0.01*
Normal rats treated with metformin	1.07 $\pm$ 0.03

All data are expressed as mean $\pm$ SEM

Disease model- Aluminium chloride treated, p value < 0.05

vs. disease model; n=6

Discussion

Insulin, not only regulates peripheral glucose homeostasis, but also has effect on learning and memory through its role on neuronal proliferation and apoptosis (16,17). It is very well established that hyperglycemia impairs cognitive function and DM is one of the risk factor for AD (6,7,8). It is also observed that about 80% of AD patients suffer from DM. Hyperglycaemia may increase oxidative stress, production of advanced glycation end products leading to structural and functional abnormalities in the brain. Since there is insulin resistance and hyperinsulinaemia, it interferes with the metabolism and clearance of amyloid beta proteins eventually leading to its accumulation and increased production of hyperphosphorylated 'tau' proteins. These are the classical hallmark of AD. DM has also been associated with brain atrophy, decreased brain weight and white matter abnormalities due to mitochondrial dysfunction, down regulation of neuronal insulin receptors and dysfunctional insulin signaling. These central nervous system changes are also seen in AD and may also lead to development of the disease.

Present study showed that step through latency (STL) and total time spent in light chamber in STZ group, were significantly less than those in control group, suggesting learning and memory impairment in the STZ group when

compared to control group. This is similar to the report by Li et al (18.) The major observation in our study is that Metformin administration is able to reverse the STZ induced altered cognitive function. Similar results are observed with the standard drug Rivastigmine also. We have seen that in STZ treated group there an increase in acetylcholinesterase activity in the hippocampal region of the brain compared to control group suggesting an impairment in cognitive function. Both Rivastigmine and Metformin treated group showed a significant decrease in acetylcholinesterase activity suggesting an improvement in learning and memory.

Due to the reported shared pathology, AD is a progressive neurodegenerative disorder which requires long-term treatment. DM is considered as a potential risk factor for cognitive impairment, dementia and AD. Outcome of our research is that metformin could serve as a potential drug for attenuating the cognitive symptoms and improving glycemic control DM and preventing associated complications. A well planned research in clinical setting may be worthwhile in finding out the beneficial effect of metformin when both the conditions co-exist. If proved useful in both the condition it may reduce the cost of treatment as treatment is lifelong in these conditions.

References

1. Perl DP. Neuropathology of Alzheimer's disease. Mt Sinai J Med. 2010; 77(1):32-42.
2. Tomasz Sobów, Marcin Flirski and Pawe, Piotr Liberski. Amyloid-beta and tau proteins as biochemical markers of Alzheimer's disease. Acta Neurobiol Exp 2004; 64: 53-70
3. Gandy S. The role of cerebral amyloid beta of Alzheimer disease accumulation in common forms. J Clin Invest. 2005; 115(5):1121-9.
4. Tokutake T, Kasuga K, Yajima R, Sekine Y, Tezuka T, Nishizawa M, Ikeuchi T. Hyperphosphorylation of Tau induced by naturally secreted amyloid- β at nanomolar

- concentrations is modulated by insulin-dependent Akt-GSK3 β signaling pathway. *J Biol Chem.* 2012; 287(42):35222-33
5. Zina Kroner, DO (2009) The Relationship between Alzheimer's disease and Diabetes: Type 3 Diabetes? *Altern Med Rev.* 14(4):373-9.
 6. Brands AM, Biessels GJ, de Haan EH, Kappelle LJ and Kessels RP (2005) The effects of type 1 diabetes on cognitive performance: a meta-analysis. *Diabetes Care,* 2:726-735.
 7. Strachan MW, Deary IJ, Ewing FM and Frier BM (1997) Is type II diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. *Diabetes Care,* 20:438-45
 8. Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS and Egan JM et al. (2008) Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus,* 18:1085-1088.
 9. Manish Nivsarkar, Aryamitra Banerjee, Deval Shah, Jaimic Trivedi, Manoj Patel, Bapu Cherian and Harish Padh (2006) Reduction in Aluminum Induced Oxidative Stress by Meloxicam in Rat Brain. *Iranian Biomedical Journal,* 10(3):151-155
 10. Frölich L, Blum-Degen D, Riederer P, Hoyer S (1999) A disturbance in the neuronal insulin receptor signal transduction in sporadic Alzheimer's disease. *Ann NY Acad Sci.* 893:290-3.
 11. Narayanan SN, Kumar RS, Potu BK, Nayak S, Bhat PG, Mailankot M (2010) Effect of radio-frequency electromagnetic radiations (RF-EMR) on passive avoidance behaviour and hippocampal morphology in Wistar rats. *Ups J Med Sci.* 115(2):91-6.
 12. Ellman GL, Courtney KD, Andres V, Jr, Feather-Stone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol,* 7:88-95.
 13. Das A, Rai D, Dikhshit M, Palit G, Nath C (2002) Effect of unpredictable stress on cognition and brain acetylcholinesterase activity in adult and aged mice. *Indian J Pharmacol,* 34:416-421
 14. Kickstein E, Krauss S, Thornhill P, Rutschow D, Zeller R, Sharkey J, Williamson R, Fuchs M, Köhler A, Glossmann H, Schneider R, Sutherland C, Schweiger S (2010) Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling. *Proc Natl Acad Sci U S A.* 107(50):21830-5.
 15. Maryam Bagheri, Mohammad-Taghi Joghataei, Simin Mohseni and Mehrdad Roghani (2011) Genistein ameliorates learning and memory deficits in amyloid beta (1-40) rat model of Alzheimers disease. *Neurobiol Learn Mem,* 95(3):270-276.
 16. Plum L, Schubert M and Bruning JC (2005) The role of insulin receptor signaling in the brain. *Trends Endocrinol Metab,* 16:59-65,
 17. Van der Heide LP, Ramakers GM and Smidt MP (2006) Insulin signaling in the central nervous system: learning to survive. *Prog Neurobiol,* 79:205-221.
 18. Li XL, Aou S, Oomura Y, Hori N, Fukunaga K and Hori T (2002) Impairment of long-term potentiation and spatial memory in leptin receptor-deficient rodents. *Neuroscience,* 113:607-15.