

Metformin ameliorates learning and memory deficits in streptozotocin-induced diabetic rats

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Article info

Received: 09 Dec 2018

Revised: 05 Feb 2018

Accepted: 12 Feb 2018

p-ISSN:2322-1895

e-ISSN: 2345-4334

Key Words:

Diabetes mellitus

Metformin

Streptozotocin

Cognition

Learning and memory

ABSTRACT

Background and Objective: Diabetes mellitus (DM) in long-term is associated with learning and memory decline. Metformin is an anti-diabetic drug with antioxidant and memory-improving effects. Thus, this research study was conducted to assess the effect of metformin on learning and memory in diabetic rats.

Materials and Methods: In the present study, male Wistar rats (n=32) were randomly assigned to 4 groups: control, control treated with metformin (200 mg/kg), diabetic, and diabetic treated with metformin (200 mg/kg). Diabetes was induced by streptozotocin (STZ) at a dose of 60 mg/kg. Metformin was administered *i.p.* at a dose of 200 mg/kg one week after STZ injection for 7 weeks. Blood sample was taken from retro-orbital plexus before STZ injection and 4 and 8 weeks after STZ injection to measure blood glucose level. Passive avoidance and Y maze tests were performed to evaluate learning and memory dysfunctions.

Results: After 8 weeks, diabetic rats showed a significant cognitive decline in passive avoidance and Y maze tests that was significantly improved after metformin treatment. In addition, metformin exerted a significant hypoglycemic effect in this model of DM.

Conclusion: This study clearly showed that treatment with metformin for 7 weeks could ameliorate cognitive decline in diabetic animals and part of its beneficial effect is due to its hypoglycemic effect.

1. Introduction

Diabetes mellitus (DM) is associated with incapacitating neurological complications in both the peripheral and the central nervous system (1, 2). DM in long term leads to some peripheral neural abnormalities like lowered motor nerve conduction velocity, axonal damage, disturbed nerve regeneration, and weakened axonal transport (3). In streptozotocin (STZ)-diabetic animals, the observed nerve damage is somewhat similar to nerve degeneration process in individuals with diabetic neuropathy (4). Different forms of neuropathies are one of the

major complications resulting in morbidity in DM patients. Pathological findings also show that DM is itself a main risk factor for dementia in Alzheimer's disease (5). Research evidence is increasing at a tremendous rate showing the effect of DM on the brain (6, 7). Passive avoidance learning and memory disturbance usually develops in STZ-diabetic rats (8, 9). Deficit of spatial memory also occurs in DM (10, 11). In addition, hippocampus-dependent synaptic plasticity is also affected in DM (12, 13). These evidences indicate that uncontrolled diabetes is associated with multiple deficits in the nervous system.

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Metformin is a safe and inexpensive medication that is available in generic form with established efficacy in treating and preventing type 2 DM via lowering peripheral glucose and insulin levels. Metformin has also recently attracted much attention due to its possibly beneficial effects on the nervous system. In this respect, metformin is capable to attenuate neuroinflammation, to protect against apoptotic cell death in cortical neurons, and to promote neurogenesis, and it is also a beneficial treatment for injured or degenerating nervous system (14-18). Therefore, this study was done to evaluate the possible efficacy of chronic metformin on alleviation of learning and memory deficits in STZ-diabetic rats using passive avoidance and Y-maze tasks.

2. Materials and Methods

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) weighing 190-230 g were housed in an air-conditioned colony room on a light/dark cycle (a temperature of 21-23°C and a humidity of 30-40%) and freely supplied with standard pelleted diet and tap water. Procedures involving animals and their care were conducted in conformity with the NIH guidelines for the care and use of experimental animals.

The rats ($n = 32$) were randomly assigned to four groups: control, metformin-treated control, diabetic and metformin-treated diabetic. The rats were rendered diabetic by a single intraperitoneal injection of 60 mg/kg STZ (SigmaAldrich, USA) freshly dissolved in cold normal saline. Diabetes was confirmed by the presence of hyperglycemia, polyphagia, polydipsia, polyuria, and weight loss. One week after STZ injection, non-fasting blood samples were collected under light ether anesthesia from retro-orbital capillary plexus and serum glucose concentrations were measured using glucose oxidation method. Only those animals with non-fasting serum glucose level higher than 250 mg/dl were selected as diabetic for the following experiments. The day on which hyperglycemia had been confirmed was designated as day 0. Metformin (SigmaAldrich, USA) was administered *i.p.* at a dose of 200 mg/kg body weight one week after STZ injection for a period of 7 weeks. Metformin was dissolved in diluted 10% Kolliphor solvent. Changes in body weight were regularly recorded during the experimental period. Behavioral tests including

passive avoidance and Y-maze were performed at the end of study in experimental groups as described below.

Short-term spatial recognition memory performance was assessed by recording spontaneous alternation behavior in a single-session Y-maze as described before (19). The maze was made of black-painted Plexiglas. Each arm was 40 cm long, 30 cm high and 15 cm wide. The arm converged in an equilateral triangular central area that was 15 cm at its longest axis. The procedure was basically the same as that described previously as follows: each rat, naive to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Arm entry was considered to be completed when the base of the animal's tail had been completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The number of maximum spontaneous alternation was then the total number of arms entered minus 2 and the percentage is calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two).

Single-trial passive avoidance test was conducted 2-3 days after Y-maze task and was according to a previous study (19). The apparatus (BPT Co., Tehran) consisted of an illuminated chamber connected to dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (5 min), the guillotine door was opened and after the rat entering the dark chamber, the door was closed and an inescapable scrambled electric shock (1 mA, 1 s once) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded and rats with ILs greater than 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as

step-through latency (STL up to a maximum of 90 s as cut-off).

All data were expressed as mean \pm S.E.M. For behavioral tests, parametric one-way ANOVA test was applied. Body weight and serum glucose levels at different weeks were analyzed using repeated measure one-way ANOVA. In all calculations, a difference at $p < 0.05$ was regarded as significant.

3. Results

After 8 weeks, the weight of the diabetic rats was found to be significantly lower versus

control group ($p < 0.01$) and metformin treatment produced no decrease in diabetic rats as compared to diabetics. In addition, diabetic rats had also an elevated serum glucose level over those of control rats ($p < 0.001$) and treatment of diabetic rats with metformin for 3 and 7 weeks caused a significant decrease in the serum glucose ($p < 0.005$) as compared to diabetics. Meanwhile, metformin treatment of control group did not produce a significant reduction regarding serum glucose level at week 8 (Fig. 1).

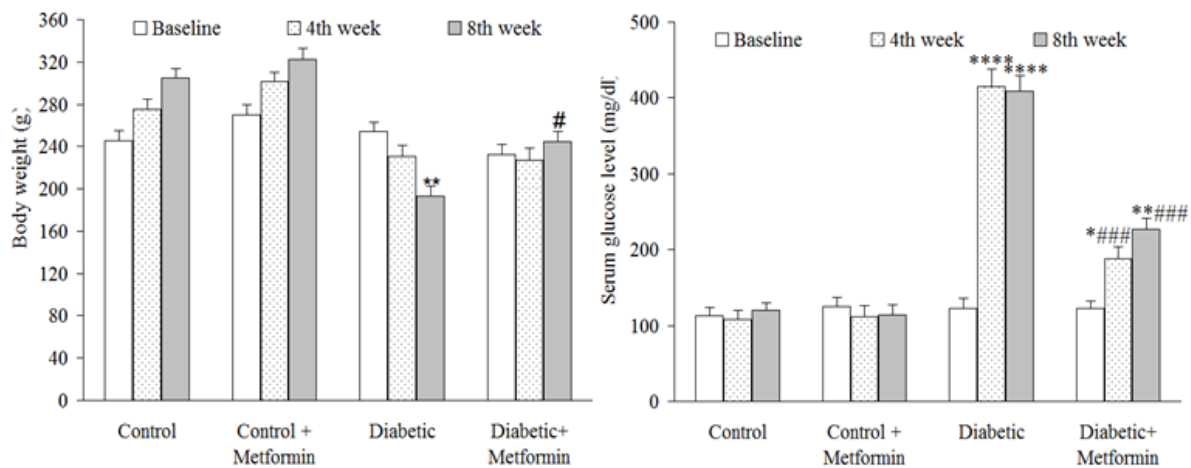


Fig. 1. Body weight and serum glucose concentration in different weeks (means \pm S.E.M)
* $p < 0.05$, ** $p < 0.01$, **** $p < 0.001$ (as compared to baseline in the same group)
$p < 0.05$, ### $p < 0.005$ (as compared to diabetics in the same week)

Figure 2 shows the results for the performance of rats in Y-maze task, in which short-term spatial recognition memory performance as alternation behavior can be tested. In this respect, the alternation score of the diabetic group was lower than that of the control ones at the end of the study ($p < 0.01$). In addition, metformin-treated diabetic group showed a higher alternation score as compared to diabetic group ($p < 0.01$) at the end of study. Meanwhile, metformin treatment of control rats did not produce any significant change regarding this parameter.

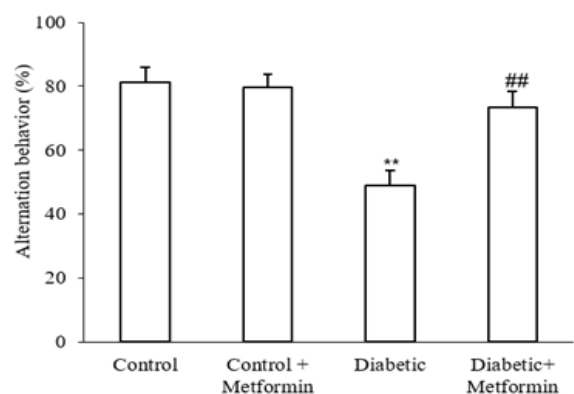


Fig. 2. Alternation behavior of treated-control and -diabetic rats in Y-maze task in different groups
** $p < 0.01$ (as compared to control)
$p < 0.01$ (as compared to diabetic)

Figure 3 shows the performance of treated-control and diabetic rats in passive avoidance task as indicated by initial (IL) and step-through (STL) latencies. Regarding IL, there was no significant difference among the groups. In addition, diabetic group showed a significant impairment in retention and recall in passive avoidance test ($p < 0.005$), as it is evident by a lower STL and metformin treatment significantly improved it ($p < 0.005$). Furthermore, retention and recall of metformin-treated control group was not significantly different from control group.

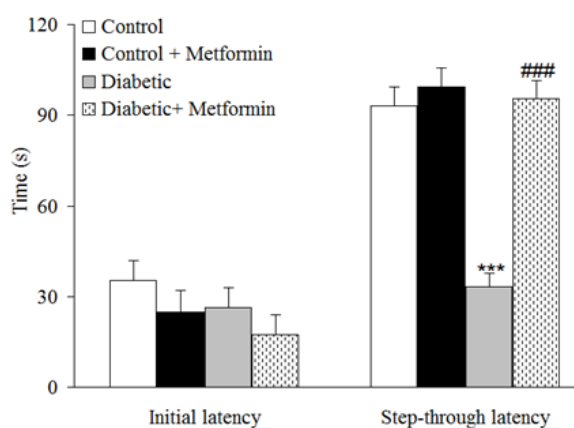


Fig. 3. Initial (IL) and step-through (STL) latencies of treated-control and -diabetic rats in single-trial passive avoidance test

*** $p < 0.005$ (vs. control)

$p < 0.005$ (as compared to diabetic)

4. Discussion

The main findings of this research were two-fold. First, chronic DM produced deficits in animal performance in passive avoidance and Y-maze tests as was verified by a lower STL and alternation score, respectively. Second, 7-week administration of metformin improved short-term spatial recognition memory performance in Y-maze and learning and memory in passive avoidance test in diabetic group.

Although the main mechanisms responsible for cognitive and memory impairments in diabetic state is still under research investigation, several factors like metabolic derangement, vascular abnormalities, oxidative stress, and neuroinflammation have been suggested (20). Chronic hyperglycemia in DM is the main cause of most of its complications. It has been known

that chronic hyperglycemia is associated with cognitive decline in DM (19, 21, 22). Thus, normalization of some cognitive abilities in diabetic group in our study may be partly attributed to the ability of metformin to reduce hyperglycemia. In addition, since brain vascular abnormalities also contributes to the pathophysiology of some cognitive impairments in DM (23-25), thus, metformin could have increased vascular endothelial nitric oxide synthase activity and improved vascular function (26, 27) and this may have been responsible for improvement of spatial recognition memory and conditioned learning and memory in our research study. Furthermore, oxidative damage is also responsible for cognitive dysfunction in DM (28, 29). Thus, treatment with antioxidants could be a therapeutic strategy in various kinds of neurodegenerative disorders (28, 29). Since oxidative stress plays a critical role in development of memory impairments in DM (28, 29), the antioxidant properties of metformin is responsible for its nootropic effects in our study (30-32). In addition, DM causes apoptosis-induced neurodegeneration in the hippocampus and to a lesser extent in the frontal cortex of rodents which is associated with cognitive decline (33). Since metformin has shown anti-apoptotic potential (34, 35), this may also have attenuated memory learning and deficits in diabetic rats in our study.

In conclusion, findings of this study demonstrated that treatment with metformin for 7 weeks could ameliorate cognitive decline in diabetic animals and part of its beneficial effect is due to its hypoglycemic effect. Further research studies are strongly warranted to explore the detailed involved mechanisms.

Acknowledgement

This work the results of M.Sc. thesis project approved and financially supported by Islamic Azad University (Science and Research Branch, Tehran, Iran) in 2016. The authors wish also to appreciate the sincere collaboration of Miss Fariba Ansari for her great technical assistance.

References

1. da Costa M, Bernardi J, Costa L, Fiuza T, Brandao R, Ribeiro MF, et al. N-acetylcysteine treatment attenuates the cognitive impairment and synaptic plasticity loss induced by streptozotocin. *Chemico-Biological Interactions* 2017;272:37-46.
2. Zou W, Yuan J, Tang ZJ, Wei HJ, Zhu WW, Zhang P, et al. Hydrogen sulfide ameliorates cognitive dysfunction in streptozotocin-induced diabetic rats: involving suppression in hippocampal endoplasmic reticulum stress. *Oncotarget* 2017.
3. Kennedy JM, Zochodne DW. Impaired peripheral nerve regeneration in diabetes mellitus. *Journal of the Peripheral Nervous System* 2005;10(2):144-57.
4. Jin HY, Liu WJ, Park JH, Baek HS, Park TS. Effect of dipeptidyl peptidase-IV (DPP-IV) inhibitor (Vildagliptin) on peripheral nerves in streptozotocin-induced diabetic rats. *Archives of Medical Research* 2009;40(7):536-44.
5. Carlsson CM. Type 2 diabetes mellitus, dyslipidemia, and Alzheimer's disease. *Journal of Alzheimer's Disease* 2010;20(3):711-22.
6. Macpherson H, Formica M, Harris E, Daly RM. Brain functional alterations in Type 2 Diabetes - A systematic review of fMRI studies. *Frontiers in Neuroendocrinology* 2017.
7. Walsh EI, Shaw M, Sachdev P, Anstey KJ, Cherbuin N. Brain atrophy in ageing: Estimating effects of blood glucose levels vs. other type 2 diabetes effects. *Diabetes and Metabolism* 2017.
8. Hasanein P, Felehgari Z, Emamjomeh A. Preventive effects of *Salvia officinalis* L. against learning and memory deficit induced by diabetes in rats: Possible hypoglycaemic and antioxidant mechanisms. *Neuroscience Letters* 2016;622:72-7.
9. Palleria C, Leo A, Andreozzi F, Citraro R, Iannone M, Spiga R, et al. Liraglutide prevents cognitive decline in a rat model of streptozotocin-induced diabetes independently from its peripheral metabolic effects. *Behavioural Brain Research* 2017;321:157-69.
10. Fatemi Tabatabaei SR, Rashno M, Ghaderi S, Askaripour M. The Aqueous Extract of *Portulaca Oleracea* Ameliorates Neurobehavioral Dysfunction and Hyperglycemia Related to Streptozotocin-Diabetes Induced in Ovariectomized Rats. *Iranian Journal of Pharmaceutical Research* 2016;15(2):561-71.
11. Wang X, Zhao L. Calycosin ameliorates diabetes-induced cognitive impairments in rats by reducing oxidative stress via the PI3K/Akt/GSK-3beta signaling pathway. *Biochemical and Biophysical Research Communications* 2016;473(2):428-34.
12. Fried PJ, Schilberg L, Brem AK, Saxena S, Wong B, Cypess AM, et al. Humans with Type-2 Diabetes Show Abnormal Long-Term Potentiation-Like Cortical Plasticity Associated with Verbal Learning Deficits. *Journal of Alzheimer's Disease* 2017;55(1):89-100.
13. Tian X, Liu Y, Ren G, Yin L, Liang X, Geng T, et al. Resveratrol limits diabetes-associated cognitive decline in rats by preventing oxidative stress and inflammation and modulating hippocampal structural synaptic plasticity. *Brain Research* 2016;1650:1-9.
14. Alzoubi KH, Khabour OF, Al-Azzam SI, Tashtoush MH, Mhaidat NM. Metformin Eased Cognitive Impairment Induced by Chronic L-methionine Administration: Potential Role of Oxidative Stress. *Current Neuropharmacology* 2014;12(2):186-92.
15. Huang YC, Hsu CC, Lin WC, Yin TK, Huang CW, Wang PW, et al. Effects of metformin on the cerebral metabolic changes in type 2 diabetic patients. *Scientific World Journal* 2014;2014:694326.
16. Mousavi SM, Niazmand S, Hosseini M, Hassanzadeh Z, Sadeghnia HR, Vafae F, et al. Beneficial Effects of *Teucrium polium* and Metformin on Diabetes-Induced Memory Impairments and Brain Tissue Oxidative Damage in Rats. *International Journal of Alzheimers Disease* ;2015:493729.
17. Tikoo K, Sharma E, Amara VR, Pamulapati H, Dhawale VS. Metformin improves metabolic memory in high fat diet (HFD)-induced renal dysfunction. *The Journal of Biological Chemistry* 2016, in press.
18. Zhang E, Guo Q, Gao H, Xu R, Teng S, Wu Y. Metformin and Resveratrol Inhibited High Glucose-Induced Metabolic Memory of Endothelial Senescence through SIRT1/p300/p53/p21 Pathway. *PLoS One* 2015;10(12):e0143814.
19. Ahshin-Majd S, Zamani S, Kiamari T, Kiasalari Z, Baluchnejadmojarad T, Roghani M. Carnosine ameliorates cognitive deficits in streptozotocin-induced diabetic rats: Possible involved mechanisms. *Peptides* 2016;86:102-11.
20. Berezin A. Metabolic memory phenomenon in diabetes mellitus: Achieving and perspectives. *Diabetes & Metabolic Syndrome* 2016;10(2 Suppl 1):S176-83.
21. Baluchnejadmojarad T, Kiasalari Z, Afshin-Majd S, Ghasemi Z, Roghani M. S-allyl cysteine ameliorates cognitive deficits in streptozotocin-diabetic rats via suppression of oxidative stress, inflammation, and acetylcholinesterase. *European Journal of Pharmacology* 2017;794:69-76.
22. Kalalian-Moghaddam H, Baluchnejadmojarad T, Roghani M, Goshadrou F, Ronaghi A. Hippocampal synaptic plasticity restoration and

- anti-apoptotic effect underlie berberine improvement of learning and memory in streptozotocin-diabetic rats. *European Journal of Pharmacology* 2013;698(1-3):259-66.
23. Hardigan T, Ward R, Ergul A. Cerebrovascular complications of diabetes: focus on cognitive dysfunction. *Clinical Science (London, England: 1979)* 2016;130(20):1807-22.
 24. Shinohara M, Sato N. Bidirectional interactions between diabetes and Alzheimer's disease. *Neurochemistry International* 2017;108:296-302.
 25. Testa R, Bonfigli AR, Prattichizzo F, La Sala L, De Nigris V, Ceriello A. The "Metabolic Memory" Theory and the Early Treatment of Hyperglycemia in Prevention of Diabetic Complications. *Nutrients* 2017;9(5).
 26. Yu JW, Deng YP, Han X, Ren GF, Cai J, Jiang GJ. Metformin improves the angiogenic functions of endothelial progenitor cells via activating AMPK/eNOS pathway in diabetic mice. *Cardiovascular Diabetology* 2016;15:88.
 27. Yue R, Fu W, Liao X, Lan C, Liao Q, Li L, et al. Metformin promotes the survival of transplanted cardiosphere-derived cells thereby enhancing their therapeutic effect against myocardial infarction. *Stem Cell Research & Therapy* 2017;8(1):17.
 28. Sharifzadeh M, Ranjbar A, Hosseini A, Khanavi M. The Effect of Green Tea Extract on Oxidative Stress and Spatial Learning in Streptozotocin-diabetic Rats. *Iranian Journal of Pharmaceutical Reserach* 2017;16(1):201-9.
 29. Wang M, Yan W, Liu Y, Hu H, Sun Q, Chen X, et al. Erythropoietin ameliorates diabetes-associated cognitive dysfunction in vitro and in vivo. *Scientific Reports* 2017;7(1):2801.
 30. Diniz Vilela D, Gomes Peixoto L, Teixeira RR, Belele Baptista N, Carvalho Caixeta D, Vieira de Souza A, et al. The Role of Metformin in Controlling Oxidative Stress in Muscle of Diabetic Rats. *Oxidative Medicine and Cellular Longevity* 2016;2016:6978625.
 31. Smieszek A, Strek Z, Kornicka K, Grzesiak J, Weiss C, Marycz K. Antioxidant and Anti-Senescence Effect of Metformin on Mouse Olfactory Ensheathing Cells (mOECs) May Be Associated with Increased Brain-Derived Neurotrophic Factor Levels-An Ex Vivo Study. *International Journal of Molecular Science* 2017;18(4).
 32. Yang X, Ding H, Qin Z, Zhang C, Qi S, Zhang H, et al. Metformin Prevents Renal Stone Formation through an Antioxidant Mechanism In Vitro and In Vivo. *Oxidative Medicine and Cellular Longevity* 2016;2016:4156075.
 33. Rahmeier FL, Zavalhia LS, Tortorelli LS, Huf F, Gea LP, Meurer RT, et al. The effect of taurine and enriched environment on behaviour, memory and hippocampus of diabetic rats *Neuroscience Letters* 2016;630:84-92.
 34. Chen B, Teng Y, Zhang X, Lv X, Yin Y. Metformin Alleviated Abeta-Induced Apoptosis via the Suppression of JNK MAPK Signaling Pathway in Cultured Hippocampal Neurons. *BioMed Research International* 2016;2016:1421430.
 35. Chen D, Xia D, Pan Z, Xu D, Zhou Y, Wu Y, et al. Metformin protects against apoptosis and senescence in nucleus pulposus cells and ameliorates disc degeneration in vivo. *Cell Death & Disease* 2016;7(10):e2441.