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Neuroprotective role of antidiabetic drug metformin against amyloid β peptide-induced neuronal loss in hippocampal CA1 pyramidal neurons in rats fed high fat diet

Masoumeh Asadbegi[1], Iraj Salehi[2], Parichehreh Yaghmaei[1], Azadeh Ebrahim-Habibi[3,4], Alireza Komaki[2*]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

[2]Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.

[3]Biosensor Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

[4]Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

***Corresponding author: E-Mail: alirezakomaki@gmail.com, Komaki@umsha.ac.ir**

ABSTRACT

Three pathological hallmarks of Alzheimer's disease (AD) include extracellular senile plaques, intracellular neurofibrillary tangles and neuron loss in the hippocampus and the associated neocortex. Metformin (Met) is able to exert an effective neuroprotective effect and also is used to treat neurodegenerative disorders such as AD. On the contrary, overweight and high fat diets (HFDs) are known to increase the risk of AD. Here, we investigated the neuroprotective effects of Met on β-amyloid (Aβ)-induced deficiencies in density of hippocampal CA1pyramidal neurons in AD model rats that were fed a HFD.

In the current study, 32 adult male Wistar rats were randomly assigned to four groups (n=8): group 1 (control); group 2 (HFD+vehicle); group 3 (HFD+Aβ); or group 4 (Met+HFD+Aβ). Rats pretreated with Met along with feeding an HFD for 8 weeks, and then Aβ injected intrahippocampally in order to induce AD. The numbers of the living hippocampal CA1 pyramidal cells were numbered as neuronal mass. The results showed that numbers of pyramidal neurons in rat hippocampal CA1 subfield were significantly decreased in Aβ-injected rats fed an HFD compared to control and vehicle-injected animals. Met pretreatment along with HFD consumption in Aβ-injected rats significantly attenuated these decreases, suggesting that Met decreased the effects of Aβ on neuron loss and presented neuroprotective effect. These findings suggest that Met pretreatment is neuroprotective against the detrimental effects of Aβ and HFDs on hippocampal neuron survival.

KEY WORDS: Alzheimer's disease, β-Amyloid, Metformin, High-fat diet, hippocampal CA1pyramidal neuron.

1. INTRODUCTION

Alzheimer's disease (AD) is an ongoing neurodegenerative condition. It is the most common cause of dementia (Heggland, 2015; Ghannad, 2016). The neuropathological features of AD comprise of the extracellular growth of amyloid-beta (Aβ) protein into neuritic plaques, hyperphosphorylation of tau-protein to form neurofibrillary tangles within neuron cells (in the hippocampus and cortex) (Peng, 2013; Morris and Tangney, 2014), neuron loss (in the hippocampus and the associated neocortex) (Xu, 2014), synapse loss, and brain atrophy (Morris and Tangney, 2014). In brain tissue, AD is likewise related with alterations in signaling cascades, gene expression, reorganization of connectivity, and synaptic dysfunction (Neuman, 2015).

The precise etiology and pathogenesis of AD remain unclear (Peng, 2013). There are several factors that can upturn AD risk including diabetes, stroke, atherosclerosis, and overweight (Knight, 2014). Human overweight is on the rise (Moy and McNay, 2013). Increasing evidence suggests that overweight can cause neuronal damage (Kim, 2015), long-term memory loss (Komaki, 2015), and synaptic plasticity impairment (Karimi, 2013; 2015). The occurrence of AD is larger in states where the consumption of high-fat/high calorie diets is high (Martin, 2014). Morbid overweight in the human is related with a growth in the expression of amyloid precursor protein (APP) (Ghanim and Dandona, 2015). High-fat diet (HFD) in animal models of AD is related with increased accumulation of the toxic Aβ peptide and impaired behavior (Barron, 2013).

Metformin (Met) is one of the most commonly used insulin sensitizer against peripheral insulin resistance (Gupta, 2011). Met is widely used to treat type II diabetes and other metabolic syndromes (Esmaeili and Khodadadi, 2012; Rasolabadi, 2015). Met, outside its anti-hyperglycemic role, can likewise perform as a pharmacological agent in the management of neurodegenerative conditions. This substance showed neuroprotective effects in various models of toxicity (Ullah, 2012). Furthermore, it has been revealed to increase neurogenesis, spatial memory formation and reduce the risk of Parkinson's disease (Esmaeili and Khodadadi, 2012). Amongst diabetic patients, long-term treatment with Met may reduce the risk of cognitive decline (Mihara and Shibamoto, 2015). Bearing in mind the connection of pharmacological agents in neuronal loss and the potential role of these substances in AD therapeutics, we investigated the potential neuroprotective effects of Met as treatment or protective compound in Aβ-induced neuronal death of rats that fed HFD.

2. MATERIALS AND METHODS

Animals: Our experiments were conducted on 32 male Wistar rats (120 ± 5 g; Pasteur Institute of Iran, Tehran, Iran). The animals were housed four to a cage in an animal room that was maintained at 20–25°C with 50–70% relative humidity under a 12-h light–dark cycle. The rats had free access to food (rodent pellets) and water. All of the study and animal care processes were approved by the Veterinary Ethics Committee of the Hamadan University of Medical Science and were accomplished consistent with Guidelines of the National Institutes of Health on the principles of laboratory animal care (NIH Publication 80-23, 1996).

Diet composition: A standard laboratory rodent chow diet was utilized for the control diet. By weight, it consisted of 23% protein, 47% carbohydrate, 5% lipids, 5% cellulose, 20% water, and vitamins and minerals (Belabed, 2006; Mobley, 2015). The control diet had a caloric density of approximately 3.0 kcal/g (Sant'Diniz, 2005; Komaki, 2015). The standardized HFD used in this study consisted of the subsequent hypercaloric constituents: 15 g of laboratory animal chow, 10 g of roasted ground nuts, 10 g of milk chocolate, and 5 g of sugar cookies (Estadella, 2004; De Melo, 2010; Karimi, 2015). These ingredients were ground and prepared in pellets that contained, by weight, 20% protein, 48% carbohydrates, 20% lipids, 4% cellulose, and 5% vitamins and minerals. The net energetic content of this diet was 21.40 kJ/g. To avoid auto-oxidation of the fat components, the food was stored at ~20°C (De Melo, 2010).

Experimental design: The rats were allowed to acclimate for seven days prior to their use in the studies. The rats were randomly assigned to four groups (n = 8 rats/group): Group 1 [Control; consumed an ordinary diet (was undisrupted)], Group 2 [vehicle group or HFD + phosphate-buffered saline (PBS); consumed a HFD for 8 weeks and then PBS], Group 3: (HFD + Aβ; consumed HFD for 8 weeks and then received Aβ1-42 injections), and group 4: [Met + HFD + Aβ; simultaneously administered Met (100 mg/kg in distilled water) by oral gavage once a day with the HFD for 8 weeks, and then Aβ1-42 was injected].

Aβ injections and surgery: Aβ1-42 (100 μg; Tocris Bioscience, Bristol, UK) was dissolved in 100 μL of PBS (vehicle solution) and incubated at 37°C for 7 days prior to use. This process results in the formation of amyloid fibrils, which are neurotoxic (Yaghmaei, 2013; Asadbegi, 2016). To create an AD model, the animals were sedated with ketamine (100 mg/kg) and xylazine (10 mg/kg) and moved to a stereotaxic apparatus (Stoelting Co., Wood Dale, IL, USA). The injections were made with a 5-μL microsyringe (Hamilton Laboratory Products, Reno, NV, USA). Relative to the bregma and with the stereotaxic arm at 0°, the coordinates for the dentate gyrus (DG) were posterior -3.6; lateral ±2.3; and dorsal 3 mm (Paxinos et al., 1985; Komaki et al., 2014; Karamian et al., 2015; Salehi et al., 2015; Tahmasebi et al., 2015; Nazari et al., 2016; Tahmasebi et al., 2016). Aβ solution (2 μL) was injected into the region on both sides at a rate of over 1 μL/2 min. The sham-operated rats received a vehicle solution. The animals were given 5–7 days of recovery (Ghahremanitamadon, 2014; Zargooshnia, 2015).

Histological study: At the end of experiment, the rats were perfusion-fixed with 10% formalin under anesthesia. Paraffin sections (6 μm) were prepared and stained with hematoxylin and eosin. The figures of the living hippocampal CA1 pyramidal cells per 1 mm length were calculated as neuronal density.

Statistical Analysis: Data are presented as mean ± S.E.M and analyzed by SPSS version 22 software. Statistical analyses were performed using one-way ANOVA. Tukey multiple comparison tests were used to analyze the significance of the differences between the groups, when appropriate. $p < 0.05$ was considered significant.

3. RESULTS

Tissue certificate: In order to check the accuracy of the model, histological analysis was performed which showed that injection of Aβ was in their intended location (Fig.1), according to the atlas of Paxinos and Watson (Paxinos, 1985).

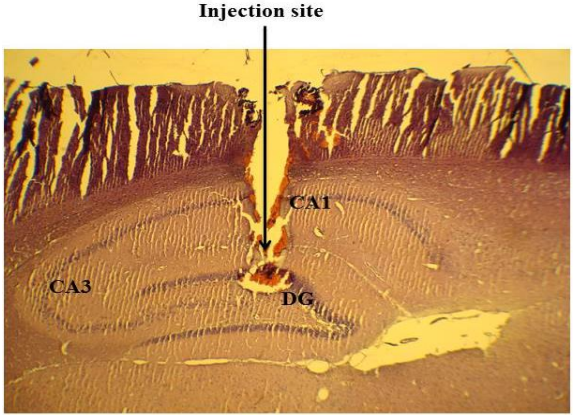

Fig.1. A typical photomicrograph illustrating the localization of the microinjection site of Amyloid β into the hippocampus sagittal section (black arrow)

Effects of metformin administration in Aβ-injected rats on body weight: Body weight in all groups was monitored throughout the period of the study. Initial weight, and final weights are shown in Fig. 2. At the beginning of the experiment, bodyweights were not significantly different among the five groups. However, over the 8 weeks, a significant difference is observed between groups. Post-hoc analysis demonstrated that HFD-fed rats had increased body weight compared with the Control group.

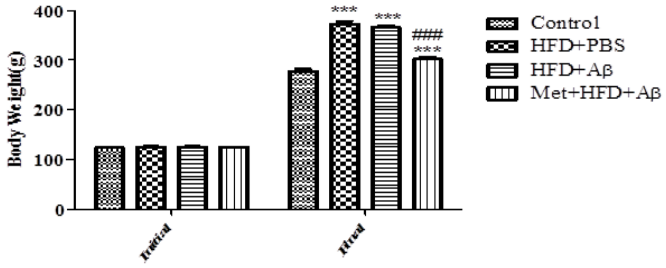

Fig.2. Body weight in all groups. Initial weight and final weights

Each column and bar represents mean ± S.E.M.*p<0.05, **p<0.01 and ***p<0.001 as compared with the control group. # p<0.05, ## p<0.01 and ### p<0.001 as compared with HFD+Aβ group.

Metformin administration confers protection against Aβ1-42-induced neuronal loss in hippocampal CA1 regions: Finally, we explore Met administration in HFD fed rats, protects against Aβ neurotoxicity in hippocampus in vivo. HE staining was used to examine the surviving pyramidal neurons in the hippocampus. Neuronal density of hippocampal CA1 region was analyzed in Fig. 3. Number of surviving pyramidal neurons hippocampus in the HFD + PBS group did not significantly differ from the control group (control: 35.13 ± 3.502, HFD + PBS: 32.38 ± 2.500; p < 0.05), But in HFD + Aβ (received Aβ1-42 injections) group, the number of surviving pyramidal neurons decreased significantly in CA1 subfield comparing with control and vehicle group (HFD + Aβ: 16.88 ± 0.8332; p < 0.001). Whereas in Met + HFD + Aβ group consumption of Met significantly promoted neuronal survival in CA1 subfield (Met + HFD + Aβ: 23.50 ± 1.680; p < 0.05) (Fig.4).

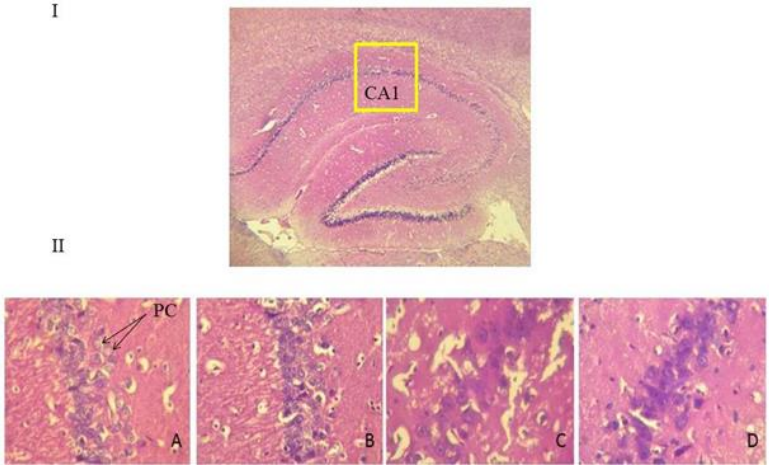

Fig.3. (I): Sagittal section photomicrograph rat hippocampus (40 ×). The numbers of the surviving hippocampal CA1 pyramidal cells per 1 mm length were counted as neuronal density. (II): Shown are representative photomicrographs (400 ×) from Control(A), HFD + PBS(B), HFD + Aβ(C), Met+HFD + Aβ(D) groups. Hematoxylin and eosin (HE) staining;PC: Normal pyramidal cells showed round and palestained nuclei. Number of CA1 pyramidal cells in the hippocampus is decreased in HFD + Aβ group as compared with the control group (p< 0.001). While, the number of CA1 pyramidal cells in the hippocampus is increased after chronic Metformin treatment (p< 0.05)

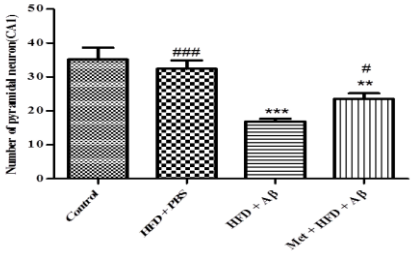

Fig.4. Effect of metformin administration on number of pyramidal cell in CA1 region hippocampus (Data present as mean ± S.E.M.)

* p <0.05, ** p <0.01 and ***p<0.001 as compared with the control group. # p <0.05, ## p <0.01 and ### p <0.001 as compared with HFD+Aβ group.

Discussion: Findings of the present study provide strong evidence of a direct neuroprotective effect of Met in Aβ-induced model of AD in animals. The number of surviving pyramidal neurons in HFD + Aβ (received Aβ1-42 injections) group, decreased significantly in CA1 subfield comparing with control and vehicle group. Consumption of Met promoted neuronal survival in CA1 subfield. AD is related with changes in the distribution, number, and size of inputs to hippocampal neurons (Neuman, 2015). Met is able to exert an effective neuroprotective effect in primary cortical neurons of cultured rats (El-Mir, 2008). It has been demonstrated that brains of HFD fed rat showed elevated levels of Tau proteins involved in APP processing and Aβ accumulation (Nuzzo, 2015). Treatment with Met prevented appearance of molecular and pathological characteristics observed in AD (El-Mir, 2008). Conversely, fatness is one of the most effective risk factor for AD in life (McNeilly, 2012). Met might interfere with the apoptotic cascade in a model of ectoposide-induced cell death by preventing penetrability transition pore (PTP) opening and obstruction of the release of cytochrome-c (Rojas and Gomes, 2013). Accordingly, Met has been shown as a neuroprotectant in contradiction to apoptotic cell death in primary cortical neurons (El-Mir, 2008).

The brain is known by high oxidative metabolism and low antioxidants enzymes, which increases the brain's vulnerability to oxidative stress (Kroemer and Reed, 2000). Oxidative stress has been implicated in a variety of neurological diseases, including AD, Parkinson's disease, and amyotrophic lateral sclerosis disease (Rojas and Gomes, 2013). Met inhibited the production of free radicals linked to the reverse electron transfer through complex I (El-Mir, 2008). In the other word, Met has antioxidant properties (Rojas and Gomes, 2013). Met treatment also prevented PKC signaling deficits and activation of NF-kappa B, mediator of cellular oxidative stress and inflammation which leads to neuronal stress and apoptosis (Ayasolla, 2005). However, little is known about Met's effects in the CNS (Yarchoan and Arnold, 2014).

Met exerts its protective effects in part by activating AMP-activated protein kinase (AMPK). The role of AMPK in many neurodegenerative diseases like cerebral ischemia, Huntington's disease and AD is well recognized (Khang, 2014; Ashabi, 2015). AMPK is triggered in the brain by metabolic stresses that hinder ATP production such as ischemia, hypoxia, glucose deprivation, metabolic inhibitors (Met), as well as catabolic and ATP consuming processes (Jun, 2008). A recent study reported that Met reduced phosphorylation of tau protein in cortical neurons of mice (Imfeld, 2012).

4. CONCLUSION

Consumption of Met results in neuroprotective effect in CA1 subfield. However, the consumption of Met does not completely reverse neuronal loss in Aβ-induced model of AD. Therefore, we suppose that pretreatment of Met by a neuroprotecctive effect can prevent from Aβ-induced cell death in pyramidal neurons of high risk rats with HFD consumption. Furthermore, these findings present a unique opportunity for prevention and treatment of AD in human.

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