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Original Article

Sitagliptin attenuates cognitive impairment in the rat model of Aluminum-induced Alzheimer's disease

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ABSTRACT

Background: Alzheimer's disease is recognized as the most prevalent form of dementia. GLP-1R agonists improve spatial memory in models of AD. This investigation was done to study the role of sitagliptin on improving memory defects in AD, induced in rats. **Methods:** 48 adult female albino rats were divided into four groups: Group I: the control normal group. Group II: received i.p. 4.2 mg/kg/day aluminum chloride for 4 weeks. Group III: treatment with 10 mg/kg sitagliptin was started 2 weeks after induction of the model for 4 weeks. Group IV: treatment with donepezil (1 mg/kg) started 2 weeks after induction of the model for 4 weeks. The NOR test and the MWM test were used. Brain amyloid beta (Aβ1–42) levels and malondialdehyde in hippocampal homogenate were assessed and histopathological examination was done. **Results:** Aluminum chloride injection in rats induced significant deterioration in cognitive function as tested with NOR and MWM tests, and a significant increase was observed in the brain MDA and Aβ1–42 levels, which was associated with the histopathological insults compared with control untreated rats. Treatment with sitagliptin significantly improved the changes of memory, reduced the brain level of MDA and Aβ, and improved the brain pathology compared with the model group. **Conclusion:** We reached a novel finding of the sitagliptin's protective effect against the neurodegenerative effect of AD induced by aluminum injection.

Keywords: Alzheimer' disease, Sitagliptin, Donepezil, Aluminum chloride, Beta-amyloid.

Introduction

Alzheimer's disease (AD) is an irreversible progressive brain disorder that causes severe impairment in cognitive function and memory as well as behavioral changes, leading to psychological symptoms like depression, anxiety, and mood disturbances [1]. Aging is the greatest risk factor for AD. Life expectancy is increasing due to advances in healthcare services, so the population at risk of AD increases. Inflammatory processes, genetic risk factors, cardiovascular disease, limited physical activity, and diabetes can contribute to age-related brain degeneration [2].

Worsening the cognitive function of AD patients correlates with the accumulation of extracellular plaques of Aβ and intracellular tangles of hyperphosphorylated Tau protein in the brain. Both types of lesions are highly toxic to neurons and predispose to dementia and neurodegeneration [3].

The reduced amount of acetylcholine in the brain is responsible for some AD symptoms, so cholinesterase inhibitors (CI) as donepezil are used for the improvement of cognitive abilities in AD patients [4].

Various animals have been used as the model of different aspects of AD. Rats were a favored species as they can make adaptive decisions and reflect their mental processes that are unique to primates [5]. Aluminum is linked with AD as it increases Aβ42 in cultured neurons of the cerebral cortex of rat [6].

Pharmacotherapy targets to prevent the accumulation of amyloid-beta in the brain. GLP-1Rs are expressed in most parts

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of the brain [7]. GLP-1 might have beneficial effects on brain physiology, learning behavior, and neuroprotection [8]. Synthetic GLP-1 analogs exert neurotrophic effect and enhance cognitive functions [9].

Sitagliptin improves cognitive function in an AD patient by inhibiting DPP-4, which is the first step in GLP-1 breakdown [10]. In addition, sitagliptin promotes the effect of BNP and stromal cell-derived factor-1α by inhibiting their degradation that may mediate the cardioprotective and neo-vascularization effect of sitagliptin [11]. Therefore, sitagliptin can control cardiovascular diseases and diabetes that may be risk factors for AD development.

This investigation was done to study the role of DPP-IV inhibitor sitagliptin on improving memory defects in the aluminum-induced model of AD compared to donepezil in rats.

Methods

Animals

48 adult female albino Sprague–Dawley rats, matched for weight and age (between 200- 230 g) were used for the experiment. During the study, each of the 2 rats were kept in one cage at room temperature on standard rat chow and fresh water ad lib. The protocol of the study was approved by the Institutional Animal Care and Use Committee, Cairo University (CU-IACUC).

Experimental design

The animals were randomly allocated into five main groups (12 rats each):

- **Group I (Control normal group):** Rats received 1ml saline daily orally until the end of the experiment.
- **Group II (Control sitagliptin treated group):** Rats received sitagliptin 10 mg/kg/day orally [12].
- **Group II (Alzheimer model group**): Rats received **aluminum chloride** (Sigma-Aldrich) 4.2 mg/kg i.p. for 28 days and left without treatment until the experiment ended [13]. The onset of AD was after 4 weeks.
- **Group III (Sitagliptin- Alzheimer group):** Rats received sitagliptin (Sigma-Aldrich) 10 mg/kg/day orally 14 days after starting aluminum chloride injection for 28 days.
- **Group IV (Donepezil-treated Alzheimer group):** Rats received donepezil (Sigma-Aldrich) 1 mg/kg/day orally daily 14 days after starting aluminum chloride injection for 28 days [14].

Morris water maze (MWM) test:

The MWM test is a swimming-based model in which animals learn to escape a pool of water by a hidden platform. The tank was equally divided into 4 quadrants. A black escape platform was submerged under the water level in the northern quadrant center. The animals were gently put in the water at one of the 4 randomly selected positions. Rats were subjected to training trials for four consecutive days. The rats were gently put in the water at one of the 4 randomly selected positions. Rats were allowed 2 minutes for locating on the submerged platform. Upon reaching the platform, rats were allowed to stay on it for 30 s. The ELT of locating the hidden platform in the water maze was as the learning index.

On day 5, the platform was removed, and the rats were allowed to explore the pool for 2 minutes. All the trials were completed approximately the same time every day (10:00 am–12:00 pm). The test was conducted and the time that the rats stayed in the target quadrant was recorded [15].

Novel object recognition test (NOR test)

In the 1st phase of the test (T1), the animals were confronted with 2 different objects in an open-field box. The time of exploring the object was registered in 5 min. After that, the animal was placed back into its home cage. In the second phase (T2), each animal was exposed to two objects, placed in the same open-field box: one familiar object, used in the first phase, and one novel object. The time spent to explore each object by the animals was recorded by using a stopwatch. The reaction of the animal to the new object was measured so that the discrimination index (DI), which is the time spent in exploring a new object over the total exploration time was calculated. As a result, the ratio of 0.5 reflected an equal exploration of the new and familiar object, which indicated no learning retention [16].

Measurement of amyloid beta (Aβ1–42) in the hippocampal homogenate

After finishing the experiment, 6 rats of each group were sacrificed by cervical dislocation after anesthesia with 50 mg/kg of ketamine i.p. Immediately, brains were excised and the hippocampus was separated and stored at deep freeze (-80°C) for later measurements. The hippocampus was homogenized in 50 mm of ice-cold sodium phosphate buffer (pH=7.4) contained 0.1 mm EDTA. After centrifugation at 1000 ×g for ten minutes at 4°C, the supernatant was used for measurement of rat brain malondialdehyde (MDA) and rat brain Aβ 1-42 [17].

Histopathological study

At the end of the test, 6 rats from each group were sacrificed by cervical dislocation after anesthesia with 50 mg/kg of ketamine i.p. and the hippocampus tissues were fixed with 10% paraformaldehyde in PBS (0.1 M, pH=7.4) for 24 hours. Brain sections (4 microns thickness) were stained by Hematoxylin and Eosin stain for histopathological examination [18].

Statistical analysis

The results were as mean ± the standard deviation of the mean. The analyses of variance (one-way ANOVA) and Post Hoc tests were used to compare the different groups. All statistics were carried out using the SPSS package (Statistical Package for Social Sciences) version 12. The differences were considered statistically as significant when p-value <0.05.

Results

MWM test

Rats of Alzheimer model group (group II) showed a 7-fold increase in latency period compared to the group I **(p<0.05)**. Treatment with sitagliptin (group III) or donepezil (group IV) led to a significant decrease in the latency period compared to group II **(p<0.05)**. The decrease in latency period observed with donepezil (group IV) was statistically significant compared to sitagliptin treated group (group III) **(p<0.05)** (**fig 1**).

NOR test

Alzheimer model group (group II) showed a highly significant decrease in discrimination index compared to group I **(p<0.05).** Treatment with sitagliptin in group III or donepezil in group IV showed a significant improvement in the discrimination index compared to DI in group II **(p<0.05).** The increase, observed with donepezil treatment was statistically significant compared to the sitagliptin-treated group (group III) **(p<0.05)** (**Table 2**).

Aβ1–42 level in the hippocampal homogenate

Alzheimer model group (group II) showed a significant increase in the rat brain Aβ level compared to group I **(p<0.05).** Treatment with sitagliptin in group III or donepezil in group IV showed a significant decrease in the amount of rat brain Aβ compared to its level in group II **(p<0.05).** There was not any significant change in the amount of brain Aβ between rats of group III and IV (**p > 0.05**) (**Table 3**).

Malondialdehyde (MDA) level in hippocampal homogenate

Alzheimer model group (group II) showed a significant increase in the amount of rat brain MDA compared to group I **(p<0.05).** Treatment with sitagliptin in group III or donepezil in group IV showed a significant decrease in the amount of rat brain MDA compared to its level in group II **(p<0.05).** The decrease observed with donepezil was statistically significant compared to sitagliptin treated group (group III) (**p<0.05**) (**Table 3**).

A strong positive correlation was between the escape latency period in the MWM test and both the brain malondialdehyde level and the brain Aβ 1- 42 for all studied groups (**p-value < 0.05**). There was a strong negative correlation between the discrimination index in NOR test and both the brain malondialdehyde level and the brain Aβ 1- 42 for all studied groups (**p-value < 0.05**) (**Figure 1**).

Histopathological results

Histological examination of the cerebral cortex and the hippocampus obtained from the control of normal rats (**group I**) revealed normal structure. The pyramidal cells of hippocampus tissue were tightly and neatly arranged, and any cell loss was not found. The cells were round and intact with nuclei, stained blue. Aluminum injection in **group II** (Alzheimer model group) caused marked histopathological damage, degeneration, and pyknosis in hippocampus neurons. In the hippocampus, a neuronal loss was region and the pyramidal layered structure was disintegrated. Neurons with shrunken cytoplasm and with pyknotic nuclei were also found (**Figure 2**).

After induction of Alzheimer (**group III**), a 28-day treatment with sitagliptin induced improvement in the degenerative changes with moderate pyknosis and mild disintegration in pyramidal layered while treatment with donepezil for 28 days (**group IV**) induced improvement in the degenerative changes with mild pyknosis and mild disintegration in pyramidal cell layered (**Figure 2**).

Table 1: Escape Latency Period in the Morris Water Maze Test

Group	Escape latency period (sec.) (Mean ± SD)
Group I (Control normal group)	10 ± 1.55
Group II (Alzheimer model group)	70 ± 9.74 [a]
Group III (Sitagliptin- Alzheimer group)	41.83 ± 4.49 [a b]
Group IV (Donepezil- Alzheimer group)	30.83 ± 2.79 [a b c]

Number of animals per each group: 12 rats

a: statistically significant compared with group I ($p < 0.05$)

b: statistically significant compared with group II ($p < 0.05$)

c: statistically significant compared with group III ($p < 0.05$)

Table 2: The Discrimination Index for Novel Object Recognition Test

Group	Discrimination index (Mean ± SD)
Group I (Control normal group)	0.84 ± 0.05
Group II (Alzheimer model group)	0.54 ± 0.02 [a]
Group III (Sitagliptin- Alzheimer group)	0.64 ± 0.02 [a b]
Group IV (Donepezil- Alzheimer group)	0.76 ± 0.02 [a b c]

Number of animals per each group: 12 rats
a: statistically significant compared with group I ($p < 0.05$)
b: statistically significant compared with group II ($p < 0.05$)
c: statistically significant compared with group III ($p < 0.05$)

Table 3: Levels of Amyloid beta (Aβ1–42) and Malondialdehyde (MDA) in Brain Hippocampal Homogenate

Group	Aβ1–42 (pg/ml) (Mean±SD)	MDA (µmol/ml) (Mean±SD)
Group I (Control normal group)	30.5 ± 7.29	4.1 ± 0.36
Group II (Alzheimer model group)	501.33 ± 79.61 [a]	20.97 ± 3.24 [a]
Group III (Sitagliptin- Alzheimer group)	110.67 ± 5.79 [a b]	15.82 ± 2.63 [a b]
Group V (Donepezil- Alzheimer group)	48.33 ± 5.65 [a b]	11.03 ± 2.2 [a b c]

Number of animals per each group: 6 rats
a: statistically significant compared with group I ($p < 0.05$)
b: statistically significant compared with group II ($p < 0.05$)
c: statistically significant compared with group III ($p < 0.05$)

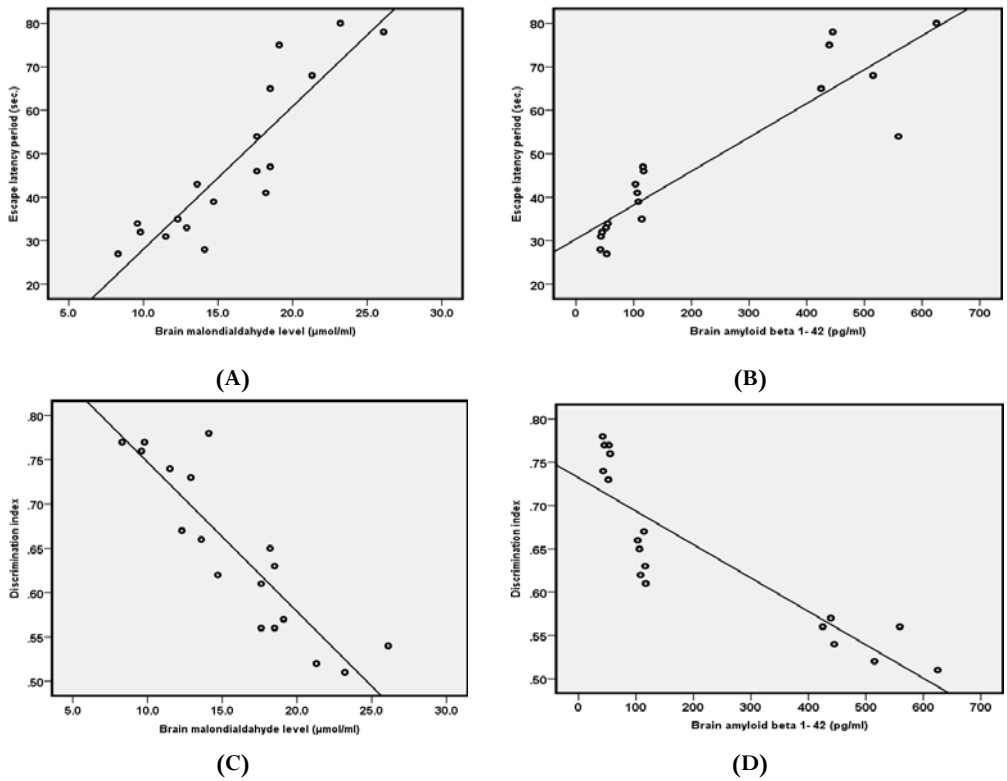

Figure 1: Correlation between the escape latency period in the MWM test and the discrimination index in NOR test with both the brain malondialdehyde level and the brain amyloid beta 1- 42

A: Strong positive correlation between escape latency period and brain MDA (p-value < 0.05)
B: Strong positive correlation between escape latency period and amyloid beta (p-value < 0.05)
C: Strong negative correlation between discrimination index and brain MDA (p-value < 0.05)
D: Strong negative correlation between discrimination index and brain amyloid beta (p-value < 0.05).

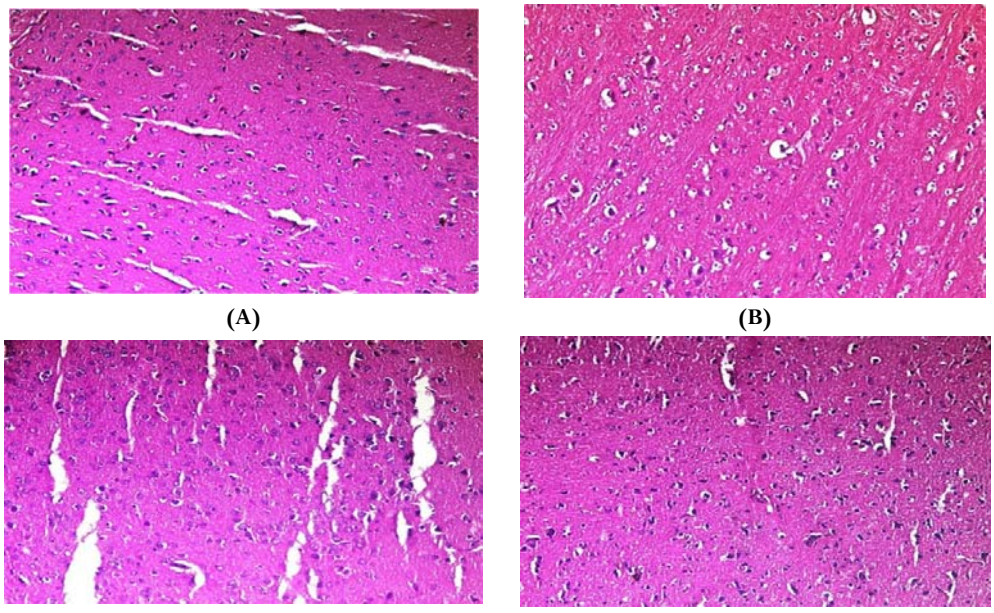

Figure 2: Brain hippocampal section (H&E x200)
A: Normal pyramidal cells without degenerative changes in the control normal group (Group I)
B: Disintegrated pyramidal cells and marked degenerative changes in Alzheimer model group (Group II)
C: Mild disintegrated pyramidal cells and moderate degenerative changes in sitagliptin-treated Alzheimer group (Group III)
D: Mild disintegrated pyramidal cells and mild degenerative changes in donepezil-treated Alzheimer group (Group IV)

Discussion

Alzheimer's disease (AD) is a prevalent cause of dementia in people over 65 years old. Despite the global increase in the incidence of AD, it is the only main cause of death, which is non-avoidable and incurable [19]. Management of the AD patient should be according to controlling the cognitive dysfunction progression as well as treatment of any associated risk factor as diabetes or cardiovascular disease [20].

This investigation was done to study the potential role of DPP-IV inhibitor sitagliptin on improving memory defects in the aluminum-induced model of AD compared to donepezil in rats.

The injection of rats with 4.2 mg/kg of aluminum for 4 weeks (**Group II**) showed deterioration in the memory and learning abilities as indicated by a significant increase in ELT in the MWM test and a significant decrease in discrimination index (DI) in the NOR test compared to the group I rats. A significant increase in the MDA level was shown in the brain homogenate study of the hippocampus, indicating oxidative stress and a significant increase in the brain Aβ 1-42 (Aβ1–42) level compared to the group I rats. Histopathological examination of brain sections showed marked damage, severe degeneration, and pyknosis in neurons of the hippocampus. There was a strong positive correlation between the escape latency period in MWM test and both the brain MDA level and the brain Aβ1–42 and strong negative correlation between the discrimination index in NOR test and both the brain MDA level and the brain Aβ1–42.

Aluminum (Al) is widely used as a non-ferrous metal. However, it is a neurotoxic agent, inducing biochemical defects in the brain by generating reactive oxygen species, which results in oxidative stress, as well as by affecting the amounts of neurotransmitters [21]. Also, the formation of neurofibrillary tangles (NFTs) and Aβ protein can be enhanced by chronic Al exposure in the AD patients' brain, which increases the number and volume of amyloid plaques [22].

Brain tissue is rich in phospholipid with a high amount of polyunsaturated fatty acids. In the case of increased free radical production, lipid peroxidation occurs. Malondialdehyde (MDA) results from the peroxidation of polyunsaturated fatty acids. MDA is a reactive aldehyde that reacts with deoxyguanosine and deoxyadenosine in DNA causing toxic stress in cells. The measurement of the amount oxidative stress in tissues as a biomarker was done by MDA [23].

Our results are consistent with the results of Shaw and Tomljenovic (2013) who investigated the aluminum neurotoxicity in animals and humans under various conditions after various administration routes and revealed that aluminum exposure led to age-related neurological deficits like AD and similar outcomes were found in animal models [24].

According to the present work, Abd-Elhady et al. (2013) induced memory changes in rats tested by open field test and passive avoidance tests through oral administration of aluminum in the dose of 10 mg/kg for 28 days that was associated with the elevation of brain MDA and oxidized glutathione, indicating oxidative stress [25]. Wang et al. (2014) found that chronic aluminum exposure followed by the disturbance of the

homeostasis of intracellular calcium and calmodulin-dependent protein kinase II are associated with impairments of memory and learning [21].

Lakshmi et al. (2015) found that the oral administration of aluminum chloride (100 mg/kg) for 21 days in rats produced a significant increase in the latency time in MWM test and a significant decrease in step-down latency in passive avoidance test that was associated with significant increase in the amount of MDA in brain tissues indicating oxidative stress [26].

Arokiasamy et al. (2015) concluded that i.p. aluminum chloride for 60 days caused a significant increase in the acetylcholine esterase activity in cortex and hippocampus and longer escape latency in MWM test with irregular hippocampal cells in the histopathological examination.

In this study, both sitagliptin and donepezil treatments for 4 weeks in rats with aluminum-induced Alzheimer disease (Group III and Group IV, respectively) showed a significant latency period decrease in MWM test, a significant DI increase in NOR test, and a significant brain Aβ1–42 and MDA decrease in the Alzheimer model group (Group II). The results were associated with histopathological improvement. Group IV treated with donepezil showed a significant improvement in the latency period in the MWM test, brain MDA level, and DI in NOR test compared to the group III that was treated with sitagliptin. While the level of brain Aβ1–42 showed no significant change between the 2 groups.

Donepezil provides the most benefit on activities, behavior, and cognition, in both severe and moderate AD. Donepezil contributes to slow down the disease progression. Petersen et al. (2005) found that donepezil significantly retarded the AD progression during the 1st year of treatment [27]. Donepezil reduces cortico-hippocampal atrophy, enhances the cholinergic neurotransmission, protects against glutamate-induced excitotoxicity, activates the neurotrophic mechanisms, and has indirect effects on cerebrovascular function by improving the brain perfusion and metabolic changes.

Donepezil acts on targets other than cholinesterases. Donepezil inhibits Na^+ currents activated by voltage [28] and up-regulates nicotinic receptors in cortical neurons [29].

GLP-1 is secreted by specific neurons in the solitary tract nucleus in the brainstem. The activation of the GLP-1R is correlated with the neurotrophic effects like neurogenesis as well as neuroprotective effects such as reduced apoptotic and necrotic signaling and cell death [30].

The present results agree with Manuela et al. (2017) who revealed that sitagliptin-induced improvement of the cognitive function, brain Aβ level, and histopathological features are through the effect of GLP-1 in the modulation of the activity of the brain-derived neurotrophic factor (BDNF). Isik et al. (2017) recorded that six-month sitagliptin therapy can improve the cognitive function in aged diabetics without and with AD [31].

Our results are consistent with Kamble et al. (2016) who detected a significant cognitive improvement of sitagliptin in rats using MWM test and Tsai et al. (2015) [32] who recorded the protective effect of sitagliptin against the cognitive impairment and brain damage in mice model of cerebral hypoperfusion. D'Amico et al. (2010) reported that orally administration of sitagliptin for twelve weeks decreased the hippocampal Aβ and the formation of amyloid plaques in the mice model for AD [33].

In the same line of the present results, Parthsarathy and Holscher (2013) showed the protective effect of liraglutide (GLP-1 analog) in AD induced in mice [34]. GLP-1 can enhance the spatial memory, increase the number of synapses in the cortex and hippocampus, reduce brain amyloid plaque load, reduce microglial activation, and increase neuronal progenitor cells [35].

Impaired brain glucose consumption and energy production in AD are linked with altered insulin and insulin growth factor-1 (IGF-1) signaling [36]. Intracerebroventricular administration of streptozotocin in mice induced memory impairment, mitochondrial dysfunction, and increase in hippocampal Aβ and hyperphosphorylated tau protein levels [37]. Insulin resistance can increase the hippocampal amyloid burden via activation of glycogen synthase kinase 3β (GSK-3β) as a consequence of attenuated insulin receptor signaling in the brain. Insulin and IGF-1 inhibit the phosphorylation of tau through the inhibition of GSK-3β and enhance the binding of tau to microtubules [38].

Sitagliptin can improve cognitive function by reducing oxidative stress [39]. Pintana et al. (2013) demonstrated that sitagliptin attenuated brain oxidative stress and restoring hippocampal mitochondrial function in rats [40]. Gault et al. (1015) and Porter et al. (2010) [41] detected that sitagliptin can reduce oxidative stress in mice with insulin resistance and obesity induced by dietary with high fat.

Sitagliptin may have a protective role on cholinergic neurons as GLP-1 receptor agonist, exendin-4, was detected to improve the activation of cholinergic neurons and preserve the cholinergic neurons in the spinal cord of mouse model with amyotrophic lateral sclerosis [42].

Conclusions

Sitagliptin attenuates the neurodegenerative changes induced by aluminum. This effect may be through improved glucose tolerance, mitochondrial function, insulin resistance, cholinergic transmission, and reduction of brain oxidative stress.

Sitagliptin has an advantage over cholinesterase inhibitors that sitagliptin can decrease the risk of development of AD through protection from diabetes and cardiovascular insult so using sitagliptin in a diabetic aged patient may decrease the incidence of Alzheimer's disease (AD) development. In addition, sitagliptin in AD patients may decrease cognitive dysfunction progression and attenuate diabetic and cardiovascular risk factors.

Therefore, DPP IV inhibitors may provide a promising way for AD management. More studies should be done to evaluate the sitagliptin's protective effect in other animal models of AD as well as its effect and safety in AD patients, especially who suffer from other diseases as diabetes and cardiovascular diseases.

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Abbreviations:

AD: Alzheimer's disease
Aβ: Amyloid Beta
Aβ42: Amyloid Beta-42
BNP: Brain Natriuretic Peptide
CI: Cholinesterase Inhibitors
DI: Discrimination Index
DPP-4: Dipeptidyl Peptidase-4
EDTA: Ethylene Diamine Tetra Acetic Acid
ELT: Escape Latency Time
GLP-1: Glucagon-like Peptide-1
GLP-1R: GLP-1: Glucagon-Like Peptide-1 receptor
GSK-3β: Glycogen Synthase K 3β
HED: Human Equivalent Dose
i.p.: Intraperitoneal
IGF-1: Insulin Growth Factor-1
MDA: Malondialdehyde
MWM test: Morris water maze test
NFTs: Neurofibrillary Tangles
NOR test: Novel Object Recognition test
PBS: phosphate buffer solution

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