Vildagliptine protects SH-SY5Y human neuron-like cells from A β 1–42 induced toxicity, in vitro

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Abstract The amyloid β (A β) toxic fibrils is thought to play a central role in the onset and progression of Alzheimer's disease (AD) because of it is a main formation of senile plaques. Diabetic patients are more vulnerable to caught Alzheimer's disease. Vildagliptine, a novel anti diabetic agent, has been reported to exert protective effects on AD rat models in restricted study. We aimed to investigate any protective effects of vildagliptine against A β fibrils on SH-SY5Y cell line. Vildagliptine decreased PSEN1 and PSEN2 mRNA levels which enroll A β production. In addition, vildagliptin was downregulated caspase-3 and caspase-9 expression levels which were evoked by A β . Also we confirmed cellular viability with real time cell analyzer and MTT assay. Our data exposed that vildagliptine has lowering effect on GSK3 β and Tau phosphorylation. However we did not get protective effect of vildagliptine against A β toxicity on mitochondrial membrane potential. These results indicate that vildagliptine exerts a protective effect against A β by decreasing apoptosis related proteins, lowering GSK3 β and Tau phosphorylation levels in addition to expression of PSEN1 and PSEN2 mRNA downregulation effect.

Keywords Cell viability · Vildagliptine · Amyloid beta · Apoptosis · Memantine

Abbreviations

AD	Alzheimer's disease
A β	Amyloid beta
Vilda	Vildagliptine
Mem	Memantine
GSK3 β	Glycogen synthase kinase 3 beta
APP	Amyloid precursor protein
PSEN1	Presenilin 1
PSEN2	Presenilin2

Introduction

Alzheimer's disease (AD) is a common neurodegenerative illness, accounting for approximately 70% of global dementia cases and leads to many cognitive and behavioral impairments. Acetylcholine esterase inhibitors such as rivastigmine, tacrine, galantamine, donepezil and N-methyl-D-aspartate receptor antagonist memantine are the only drugs approved by the US Food and Drug Administration (FDA) for the palliative remedy of AD (Reitz and Mayeux 2014).

The precise etiology of AD is still unknown. But also owing to its multifactorial nature, numerous mechanisms plays role on the progression of AD. Some characteristic features of AD are Amyloid beta

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(A β) accumulation, increment of the phosphorylated Tau/Tau level, neurofibrillary tangles, senile plaques and increased activation of prodeath genes and related signaling pathways such as capsases family (Krajewski et al. 1999; Spires-Jones and Hyman 2014). Also up regulation of APP, PSEN1 and PSEN2 expressions, impaired energy metabolism, mitochondrial dysfunction, chronic oxidative stress and DNA damage plays an important role on AD (Atrakchi-Baranes et al. 2017; Rivera et al. 2005).

Epidemiological studies have been reported that there is strong link between diabetes and AD-type neurodegeneration (Luchsinger et al. 2001). This relationship is so strong that a group of scientist suppose; AD is Type3 diabetes (de la Monte and Wands 2008). Some of the complications of Type 2 Diabetes mellitus (T2DM) such as cognitive dysfunctions, brain insulin resistance, oxidative stress, and energy metabolism defects are also symptoms of AD (Anand et al. 2014; Craft 2006; Steen et al. 2005). Especially epidemiologic studies reported that late onset AD is markedly associated with and T2DM because of the diabetic people are more vulnerable for AD than non-diabetic ones (Haan 2006). Accumulating evidence suggests that diabetes related AD is associated with metabolic dysfunction in the brain (De Felice and Ferreira 2014), deficiencies cellular energy metabolism (Carvalho et al. 2015), abnormal GSK3 β activation (Cormier and Woodgett 2017), hyperphosphorylated tau (Hernandez et al. 2013) and the apoptotic signaling pathways (Krajewski et al. 1999; Sadeghi et al. 2016). However, the underlying mechanisms and potential prevention methods have not to be elucidated completely yet.

Recent effective treatment strategy in T2DM is the use of incretin-based therapies based on the stimulation of Glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) linked pathways. GLP-1 and GIP are the two primary incretin hormones secreted from the intestine and then they increase insulin secretion from pancreatic beta cells (Fosgerau and Hoffmann 2015). GIP and GLP-1 exert their effects by binding to their specific receptors the GIP and the GLP-1 receptor and increases the level of intracellular cAMP in pancreatic β cells, thereby stimulating insulin secretion with glucose-dependently. This endogen hormones are deactivated by dipeptidyl peptidase 4 enzyme (DPP-4) (Prasad-Reddy and Isaacs 2015; Scheen 2010).

Vildagliptine is one of the DPP-4 inhibitor, has been using treatment of DM since 2007, which exerts its effect with increasing GIP and GLP-1 endogen hormones. Indeed this drug has been demonstrated that has positive effect on cognitive functions (Tasci et al. 2013) and improve insulin sensitivity and brain mitochondrial dysfunction, also decrease brain mitochondrial ROS production on high-fat diet induced insulin-resistant rats (Pipatpiboon et al. 2013). In another preclinical study showed that; vildagliptine decreased p-Tau, A β levels on the STZ induced AD model rats, also IL-1, IL-6 and TNF- α levels, which are inflammation markers, elucidated an ameliorative effect comparing to STZ group. Besides that, this drug showed positive impairment on cognitive functions in the same study (Kosaraju et al. 2013a, b).

Although DPP-4 inhibitors and GLP-1 agonist drugs are showed protective effects on AD biomarkers and behavioral studies in preclinical studies (Pintana et al. 2013), potential mechanisms underlying these effects remains to be studied. The purpose of the present study was to explore whether vildagliptin was able to prevent from A β induced toxicity in the SH-SY5Y cells. So we investigate caspase-3, caspase-9, caspase-12 to uncover if vildagliptine has any effect on cellular apoptosis. Also AD associated parameters are p-Tau/Tau and p-GSK3 β /GSK3 β expression levels researched to understand potential neuroprotective effect against. As well APP, PSEN-1 and PSEN-2 genes expression, have several effects on AD progression and A β production, levels measured. In addition MMP and NO levels determined which are playing role neural survival.

Methods

Cell culture

SH SY5Y cell line was purchased from the American Type Culture Collection (ATCC). The cells were grown in Dulbecco's Modified Eagle's Medium-F12 medium enriched with 10% fetal bovine serum, 2 mM L-glutamine and 1% penicillin/streptomycin. Cells incubated in a humidified atmosphere containing 5% CO₂ at 37 °C. When the confluence nearly reached 80%, the cells were washed with Dulbecco's Phosphate-Buffered Saline (DPBS) solution and detached from the flasks with trypsin/EDTA. The cells were

centrifuged at 1000 rpm for 5 min at 25 °C, seeded on 6 wells plate, 96 wells plate and 96 wells E-plate for western blot, MTT and xcelligence analysis, respectively.

Preparation of A β peptides

Formation of A β (1–42) peptide fibrils was carried out according to protocol described by Karie N. Dahlgren et al. (2002). In brief A β (1–42) peptide was initially dissolved to 1 mM in hexafluoroisopropanol and separated into aliquots in sterile micro centrifuge tubes. Hexafluoroisopropanol was removed under vacuum, and the peptide film was stored desiccated at – 20 °C. The peptide was first suspended in dry DMSO to a concentration of 5 mM. To obtain fibril formation of A β for applicate to further experiments, 10 mM HCl was added to bring the peptide to a final concentration of 100 μ M and incubated for 24 h at 37 °C. Than A β fibril formation treated to cells for further analysis.

MTT assay

Cells were seeded onto 96-well plates at 12,500 cells/well and treated with vildagliptine (250, 100, 50 μ M) and memantine (100 μ M) in the presence of A β (5 μ M) for 24 h. After 24 h, MTT solution (final concentration, 0.5 mg/mL; Sigma-Aldrich) was added and incubated with the cells for 4 h. The supernatant was carefully removed and 100 μ l DMSO (Sigma-Aldrich) was added to fully dissolve the crystals. OD values were measured at 490 nm using a micro plate reader (ELX800; BioTek Instruments, Inc., Winooski, VT, USA).

Xcelligence assay

Xcelligence was performed as described by Wang et al. (Ke et al. 2011) with slight modifications. The electrical impedance was measured by the RTCA integrated software of the xcelligence system as a dimensionless parameter termed cell index (CI). Cells were grown and impedance was measured every 15 min. According to growth curve and CI values 12,500 cells/well were selected for further analyzes. 12,500 cells/well were seeded in the E-plate 96 wells and 24 h post-seeding when the cells were in the log growth phase, the cells were treated with different

concentration of vildagliptine (250, 100, 50 μ M) and memantine (100 μ M) in the presence of A β (5 μ M).

Measuring of mitochondrial membrane potential assay

The mitochondrial membrane potential of SHSY-5Y cell line was measured using the Cayman's JC-1 mitochondrial membrane potential assay kit (Cayman, Cat. No. 10009172) following instructions provided by the manufacturer. MMP kit utilizes a lipophilic cationic dye (JC-1) that accumulates in functional mitochondria according to its membrane potential. In healthy cells JC-1 forms J-aggregates which gives red fluorescent (excitation/emission = 540/570 nm). The apoptotic and unhealthy cells gets into monomers which gives green fluorescence (excitation/emission = 485/535 nm). The ratio of fluorescence intensity of aggregates to monomers used as an indicator of cell health. Cells were cultured in 96 black well plates and treated with memantine (100 μ M) and vildagliptine (250, 100, 50 μ M) in the presence of A β (5 μ M) at 37 °C for 24 h. 100 μ l of the JC-1 staining solution per mL of culture medium was added to each well of the plate and mixed gently. Data was recorded at BioTek Gen5 multifunctional system (BioTek, US).

RNA extraction, reverse transcription and real-time PCR (RT-qPCR)

At the 24 h post-treatment of molecules, supernatant removed and wells washed with PBS than total RNA was isolated using RNeasy isolation reagent (Sigma-Aldrich, St. Louis, MO), from the cells according to the manufacturer's instructions. Total RNA (1 μ g) was reverse-transcribed to cDNA using a Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). Real-time PCR was performed with using a Light Cycler 480 System (Roche Diagnostics GmbH, Mannheim, Germany). To quantify cDNA, qPCR was performed using FastStart Essential DNA probe master mix (Roche Diagnostics GmbH, Mannheim, Germany) and catalogue assay kit (kits consisting the primers and probes for determination of PSEN1, PSEN2, APP and β -actin). The qRT-PCR sequences were as: APP; 5'-TCCGA-GAGGTGTGCTCTGAA-3' (Forwar) and 5'-CCA-CATCCGCCGTAAAAGAATG-3' (Reverse), PSEN1; 5'-TGCACCTTTGTCCTACTTCCA-3'

(Forward) and 5'-GCTCAGGGTTGT-CAAGTCTCTG-3' (Reverse), PSEN2; GAGCT-GACCCTCAAGTATGG -3' (Forward) and GTGAAGGGCGTGTAGATGAG-3' (Reverse) and human β -actin used for normalization. For each sample, the level of target gene transcripts was normalized to β -actin. Cp values were calculated using the software of the Roche Light Cyclor 480. $2^{-\Delta\Delta CT}$ values was used for relative quantification.

Western blotting

Western blot analysis was carried out using crude lysates of SH SY5Y human neuroblastoma cells. Cells were treated with vildagliptine 250, 100, 50 μ M; memantine 100 μ M; concentrations for 24 h both. Cells were lysed in commercial rita lysis buffer (santa cruz sc-24948). Protease inhibitor cocktail containing leupeptin, pepstatin A, chymostatin, and aprotinin (1 mg/mL). The lysate was centrifuged at 4 °C for 30 min at 12,000 rpm. Protein content in the samples was measured using the cell signalling protein dye assay reagent. 30 μ g protein lysates were resolved on 12% sodium dodecyl sulphate (SDS)-polyacrylamide gels. Then electro-transferred onto polyvinylidenedifluoride (PVDF) membrane. After blocking with 5% non-fat milk in Tris-buffered saline (TBS, 0.1 M, pH 7.4). Membrane were incubated with primary antibodies; caspase-3 (1:1000, cleaved, Biovision), caspase-9 (1:1000, cleaved, Biovision), caspase-12 (1:1000, Abcam), Tau (1:1000 CST), p-Tau (Ser202) (1:1000, CST) p- GSK3 β (ser9) (1:500 dilution, Cell Signalling Technology (CST)), GSK3 β (1:1000 dilution, CST), GAPDH (1:1000, CST) and β -actin (1:2000, CST).

β -actin and GAPDH protein was assigned as a control for protein loading. After overnight incubation at 4 °C conditions, membranes were incubated with secondary antibody, HRP-conjugated goat/rabbit anti-IgG, for 1 h at room temperature. After each step blots were washed three times with Tween (0.2%)-Tris-buffer saline (TBST). Protein bands were detected by enhanced chemiluminescence method (ECL, Santa Cruz Biotechnology, CA) on Bio-Rad imaging system. The blots were scanned and analyzed using ImageJ software.

Measurement of NO⁻ level with Griess Reagent

NO levels in cell-culture supernatants were measured with Griess reagent using a nitricoxide assay kit (Sigma-Aldrich, Germany) according to the manufacturer's protocol. In this method, nitrite is first treated with a diazotizing reagent, sulfanilamide (SA), in acidic media to form a transient diazonium salt. This intermediate is then allowed to react with a coupling reagent, N-naphthyl-ethylenediamine (NED), to form a stable azo compound. Absorbance was measured at 540 nm using a plate reader (BioTek, US).

ATP level determination

ATP level was measured according to manufacturer's instructions (Abcam, ab113849). ATP kit was used to determine level of ATP. Principle of this assay is bioluminescent methods which use an enzyme named luciferase, catalyses the formation of light from ATP, subsequently emitted light read by using a fluorescent plate reader (Biotek, Synergy HT).

Statistical analysis

All values are expressed as the mean $\pm$ standard deviation. The data were analyzed using Graphpad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Comparisons between two groups were performed using a one-way analysis of variance, whereas the comparisons among multiple groups were performed with Tukey post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of vildagliptine in the presence of A β on SH-SY5Y Cell Viability

To assess possible effects of vildagliptine on SH-SY5Y cells in the absence of A β , we treated the cells with vildagliptine for logarithmic and arithmetic concentrations (Data not shown). Then we incubated the cells with vildagliptine (250, 100, 50 μ M) in the presence of A β (5 μ M). After the 24 h treatment time we determined that A β decreased cell viability comparing to control. Memantine (100 μ M) and vildagliptine

(250 μM) increased the cell viability comparing to $\text{A}\beta$ group (Fig. 1). Maximum concentration of vildagliptine showed nearly same effect with memantine which is positive control.

Xcelligence results

Cell index give information about cell proliferation, growth rate/death rate and cell adhesion capability. When cell index reached nearby 1 value, agents were treated to cells after 8 h overnight incubation. We demonstrated the real time cell analyzing graph from treatment time (Fig. 2a). $\text{A}\beta$ treated cells' cell index decreased comparing to control. Max concentration of vildagliptine and memantine increased cell index comparing to $\text{A}\beta$ treated cells. Increment of the cell index of memantine treated cells was detected highest value.

As shown Fig. 2a, Although cell index values of vildagliptin and memantine treated cells displayed parallel line with control, $\text{A}\beta$ treated cells remained under control during the 6–24 h after treatment. This decline was became evident at last hours (12–24 h). This time dependent results gives an important idea for

advanced experiments for predicting application of agents in optimum time.

Alterations of mitochondrial membrane potential and NO^- levels on $\text{A}\beta$ 1–42 induced cells

NO^- levels did not change after treatment of drugs in the presence of $\text{A}\beta$. Our data showed that 5 μM of $\text{A}\beta$ exposure is not related with NO^- alteration in the SH-SY5Y cells. Also we performed another parameter which is mitochondrial membrane potential gives an idea about neuronal survival.

To assign the function of mitochondria, we performed MMP experiment to have an idea about healthy/unhealthy cells after treatment of vildagliptine and memantine in the presence of $\text{A}\beta$. As data shown Fig. 3a, $\text{A}\beta$ decreased MMP comparing to control group. Although 250 μM concentration of vildagliptine increased MMP but this increment was not significant.

Western blotting results

Vildagliptine attenuated the $\text{A}\beta$ 1–42 induced apoptosis via caspases evoked pathway

Regarding apoptotic signaling pathways, our data demonstrated that cleaved caspase-3 which is associated caspase-9 expression, was markedly decreased by memantine and vildagliptine in a dose dependent manner. Activated caspase-9, which plays a main role in the activation of procaspase-3, was also down regulated by this drugs at the 24 after treatment time (Fig. 3). We further determined the levels of caspase-12 to ask any effect on different apoptosis pathway from caspase-3. When we look at caspase-12 expression levels, we assigned that an increment with the $\text{A}\beta$ exposure. Than vildagliptine decreased this increment dose dependent manner but this effect was not statistically meaningful. Also memantine could not change caspase-12 level markedly (Fig. 4).

Vildagliptin attenuates the deficit in the $\text{GSK3}\beta$ pathway and related parameter of Tau phosphorylation

Previous studies have demonstrated that $\text{A}\beta$ exposure causes up regulation of Tau protein phosphorylation. So, we determined expression of p-Tau/Tau whether

Fig. 1 Effect of vildagliptine and memantine on $\text{A}\beta$ induced toxicity. SH SY5Y cells were treated for 24 h with vildagliptine (250, 100, 50 μM) and memantine (100 μM) in the presence of $\text{A}\beta$ (5 μM). After that, metabolic activity was evaluated by measuring the capacity of the cells to reduce MTT. The results are presented as the percentage of absorbance determined for control conditions and represent the mean $\pm$ SD of at least three independent experiments performed in six wells (c = $P < 0.001$, significantly different from control; e = $P < 0.01$ and f = $P < 0.001$ significantly different from $\text{A}\beta$ peptide treated group)

Fig. 2 Effect of vildagliptine and memantine on A β -induced toxicity. SH SY5Y cells were treated for 24 h with 5 μ M A β in the presence of vildagliptine (250, 100, 50 μ M) and memantine (100 μ M). After that, cell index was evaluated by measuring the capacity of the cells to changing of impedance. Time dependent cell index alterations were showed (a). Cells were treated with

agents at 8 h after beginning of incubation. After 24 h treatment of the agents, the results are presented as cell index determination and represent the mean $\pm$ SD (b) of at least three independent experiments performed in six wells (a = $P < 0.05$, significantly different from control; f = $P < 0.001$ significantly different from A β peptide treated group)

vildagliptine can recover over phosphorylation of Tau. Treatment of A β increased the level of p-Tau/Tau, however maximum concentration of vildagliptine and memantine down regulated this level comparing to A β treated cells, while the 100 and 50 μ M concentrations of vildagliptine did not decreased up regulated p-Tau significantly (Fig. 5).

Alterations of the APP, PSEN1 and PSEN2 mRNA expression levels

Expression of APP, PSEN1 and PSEN2 determined with RT-qPCR. There was no significant alteration on APP mRNA levels with vildagliptine treatment (Fig. 6a). However vildagliptine suppressed PSEN1 and PSEN2 mRNA in a dose dependent manner. There was significant change in 250 and 100 μ M concentration of vildagliptine for PSEN1 and 100 μ M concentration of vildagliptine for PSEN2 (Fig. 6b, c). Our

Fig. 3 Effect of vildagliptine and memantine on $A\beta$ induced cells. NO^- levels did not change after treatment of drugs and $A\beta$ (a). MMP level decreased in the presence of $A\beta$ and this decrement did not recovered by vildagliptine and memantine, markedly (b). Highest concentration of vildagliptine over regulated ATP level on $A\beta$ induced cells, significantly (c)

positive control drug is memantine increased APP expression (Fig. 6a), while it decreased PSEN1 and PSEN2 expression levels (Fig. 6b, c).

ATP levels

Optimum level of ATP in the alive cell is related with healthy mitochondrial function represents maintaining mitochondrial membrane potential without any decrement. To interpret cellular survival action with mitochondrial function, ATP level determination is a providing factor which are both maintain cell energy metabolism. It has been showed before that decreasing of MMP level causes also downregulation of ATP (Eckert et al. 2003; Huang et al. 2005). $A\beta$ treatment to the SH-SY5Y cells was decreased ATP level, however highest concentration of vildagliptine recovered this decrement to nearby control (Fig. 3c). But our positive control drug is memantine was not successful to increasing ATP significantly, in contrast to Mansoor et al. (2010) have showed that memantine's positive increasing effect on Müller cells.

Discussion

Cognitive impairment is one of the most seen complication of diabetes and $A\beta$ plays a major role in disruption of cognition. Whereas phosphorylation rate of Tau protein is another process playing crucial role in progression of AD. Although several clinical and preclinical studies have executed for many years, unfortunately very limited drug can be ordered by clinicians to patients who are suffering from AD. When difficulties of human neural crest stem cells usage take into account, immature cells are may be scientific tool because of their neuron like phenotypes such as expressing $A\beta$, Tau, synaptophysin and other neuron specific proteins (Agholme et al. 2010).

This study demonstrated that vildagliptine showed protective effect against to $A\beta$ induced toxicity on SH-SY5Y cells. SH-SY5Y cells widely used as suitable model to explore the mechanism of degeneration of neuron cell like phenotype because of the expressing $A\beta$, tau, synaptic factors and other neuron specific proteins (Agholme et al. 2010; Lopes et al. 2010). As previous studies reported that $A\beta$ has toxic effect on neuronal cells (Jan et al. 2011; Spires-Jones and

Fig. 4 Effect of vildagliptine and memantine on A β 1–42-induced toxicity. SH SY5Y cells were treated for 24 h with 5 μ M A β 1–42 in the presence of vildagliptine (250, 100, 50 μ M) and memantine (100 μ M). Total protein lysate was separated by SDS-PAGE (4–20% acrylamide, 25 μ g/lane), electro transferred to PVDF membrane and probed with antibody to caspase-3 and caspase-9 and caspase-12. The results are presented as percent expression and represent the mean $\pm$ SD at least three independent experiments performed. (b = $P < 0.01$ significantly different from control; d = $P < 0.01$ and f = $P < 0.001$ significantly different from A β peptide treated group)

Fig. 5 Effect of vildagliptine and memantine on A β 1–42-induced toxicity. SH SY5Y cells were treated for 24 h with vildagliptine (250, 100, 50 μ M) and memantine (100 μ M) in the presence of 5 μ M A β 1–42. Total protein lysate was separated by SDS-PAGE (4–20% acrylamide, 25 μ g/lane), electro transferred to PVDF membrane and probed with antibody to Tau,

p-Tau, GSK3 β and GSK3 β . The results are presented as expression ratio and represent the mean $\pm$ SD at least three independent experiments performed. (b = $P < 0.01$ significantly different from control group; d = $P < 0.01$ and f = $P < 0.001$ significantly different from A β peptide treated group)

Fig. 6 Effects of vildagliptine (250, 100, 50 μ M) and memantine (100 μ M) on APP(a), PSEN1(b) and PSEN2 (c) in the SH-SH5Y cell line. The mRNA expression levels assigned from three independent experiment and presented as mean $\pm$ SD. (a = $P < 0.5$, b = $P < 0.01$ and c = $P < 0.001$ significantly different from control group)

Hyman 2014), here we confirmed that A β showed cytotoxic effect via caspase family, GSK activation and enhanced tau protein phosphorylation.

A β is one of the most commonly used agents to experimentally induce toxicity, and toxicity occurs in essentially neuronal cells exposed to appropriate formation of fibril form of A β (Lorenzo and Yankner 1994), suggesting that it activates a cell death program. As previous studies reported the A β toxic effect on SH-SY5Y cell line (Tarozzi et al. 2008), we collected the data with time dependent during for 48 h every 15 min. A β decreased cell index after treatment of the cells and surrounded this effect during the experiment. Vildagliptine recovered cell index to nearby control's cell index in a dose dependent manner. Although the positive effect of vildagliptine on cell viability have shown before (Dei Cas et al. 2017), we achieved this effect time dependent manner on SH-SY5Y cells and MTT results corrected this result at 24 h.

Among the most prominent of death-specific enzymes, family of cysteine-dependent aspartate-specific proteases known as the caspases, enroll both physiological apoptosis and injury-dependent apoptosis. So any therapy which manipulates caspases activity, must take into account the probable effect

on cell homeostasis. Tenneti and Lipton (2000) reported that memantine down regulates the caspase-3 expression induced by A β fibrils, we confirmed this data for memantine in addition to vildagliptine's similair effect.

It has been documented that caspase-9 is associated with the apoptotic pathway and over activity of it leads to neuronal loss (Krajewski et al. 1999). Previous studies showed that over exposure of A β upregulate caspase-9 activation (Awasthi et al. 2005). In the present study, down regulation was observed with vildagliptine and memantine treated cells in the levels of caspase-9. Also mitochondrial pathways causes caspase-9 activation whose main function is initiating apoptosis pathway (Krajewski et al. 1999). In contrast, caspase-9 is activated when cytochrome c is released into the cytoplasm from the mitochondrial membranes (Susin et al. 1999). Although caspase-9 has been reported to associated with MMP on cellular apoptosis (Green and Reed 1998; Hakem et al. 1998), we did not collect data which is about interpreting caspase-9 and MMP correlation in SH-SY5Y cell line.

Intracellular neurofibrillary tangles one of detrimental component of AD, are mainly composed of hyperphosphorylated tau. When tau is hyperphosphorylated by intracellular mechanisms excessively, its

detachment from microtubules increases and axonal transport of synaptic vesicles deteriorates. Consistent with present study Sengupta et al. (Li et al. 2004) proposed that memantine suppressed hyperphosphorylation of tau. In our study it was discovered that vildagliptine decreased p-Tau/Tau ratio. Either this result is important for fulfillment physiological microtubule activation, or related with inducing apoptotic pathway results cellular alive.

There is accumulated evidence reported that tau phosphorylation seems to be related to altered activity of kinases such as GSK3 β which is one of the intracellular enzyme, plays a role in apoptosis (Hernandez et al. 2013; Tenneti and Lipton 2000). Considering the GSK3 β /p-GSK3 β is sign of this protein activation, vildagliptine can be effective decreasing activation of GSK3 β . Also vildagliptine may down regulated hyper phosphorylation of tau because of its GSK3 β deactivation effect. Our results differ from Pap and Cooper (Pap and Cooper 1998) in that transient transfection of GSK3 β caused 60% of PC-12 and Rat-1 cells spontaneously undergo apoptosis. We achieved bigger cell viability with over activated GSK3 β . These difference may be related with selecting another neuron like cell is named SH-SY5Y.

In marked contrast to its modest beginning as a regulator of glycogen synthesis, GSK-3 β has been found to participate in a remarkable number of signaling pathways, which, based on the findings reported here, include the ultimate decision between cell death and survival. Activation of GSK3 β potentiates A β production, tau phosphorylation [34] and other cellular disruptions [35] and thus cells much more vulnerable to the apoptosis-inducing actions. So, suppressed activation of GSK3 β by vildagliptin may be taken into account for GSK3 β related apoptotic pathways. It has been reported that memantine decrease GSK3 β activity in several studies for in vivo (Ponce-Lopez et al. 2011) and in vitro (Jantas et al. 2009), we also confirmed this effect of memantine in SH-SY5Y cell line.

Mitochondrial dysfunction is an important element of the toxic effect of amyloid peptides in neuronal cells. The cytoplasmic concentration of Ca⁺² ions increases in response to A β , leading to the opening of mitochondrial pores and a drop in mitochondrial potential. Lower mitochondrial potential causes negative effect on cell survival. Rhein et al. demonstrated

that A β deteriorates the mitochondrial function in SH-SY5Y cells (Rhein et al. 2009). While decrement effect of A β on MMP levels was achieved in present study, neither vildagliptine, nor memantine did not recovered MMP levels nearby to control. In a previous study, over expression of caspase-3 and caspase-9 documented linked with MMP in HL-60 cells (Pap and Cooper 1998). When we look at caspase-3 and caspase-9 down regulation same as MMP at A β treated cells, it can be seen consistent results evaluated. Our contradictory result is that while vildagliptine decreased caspases, but did not be successful for increasing MMP. These findings supported that SH-SY5Y cells have showed different response from HL-60 cells.

Kuhla et al. (2004) have exposed that A β decrease ATP level in SH-SY5Y cells, additionally other group (Al-Mousa and Michelangeli 2012; Seoposengwe et al. 2013) investigated the MMP and ATP level, are both examined and exhibited similar response on neuroblastoma cells. Our results corrected effect of A β on ATP level, in addition vildagliptine recovered ATP in the present study. Equilibrium of cellular energy metabolism would be considered with proteins discussed in this text for interpreting cellular viability. So demonstration of up regulation with vildagliptin on MMP and ATP level is meaningful when taking into account proteins, which are discussed in this text.

Increased endoplasmic reticulum stress is one of the causative factor of caspase-12 evoked apoptosis. Caspase-12 localizes either the mitochondria or the endoplasmic reticulum and it co-localizes with several proteins such as PSEN2 and APP. APP is cleaved by caspase-3, releasing A β which, in turn, activates downstream caspases including caspase-3 and, possibly, caspase-12 to amplify the effects (Mehmet 2000). So, down regulation of caspase-12 is playing a major role in neurodegeneration also its results to apoptosis. Although vildagliptine decreased APP and PSEN2 expression levels, caspase-12 level did not downregulate by vildagliptine markedly in the present study.

APP overexpressing mice have been widely used in the study of AD. Focusing mainly at older ages, higher accumulation of A β is associated with APP. There are conflicting reports about whether the overexpression of the APP causes neurotoxicity or neurogenesis via excessive A β production (Lopez-Toledano and Shelanski 2007; Ryman and Lamb 2006). Taking into account that A β is formed as a result of the cleavage of

APP by beta and gamma-secretases, the expression of PSEN1 and PSEN2 protein involved in the structure of gamma secretase is undoubtedly important in terms of A β over-production. So downregulation effect of vildagliptine on PSEN1 and PSEN2 might lead to lower production of A β . Park et al. (2012) previously found that neurons and PC12 cells expressing wild- and mutant-type PSEN2 (N141I) increased PSEN2 expression and generation of amino acids. While PSEN1 and PSEN2 expression level was demonstrated in our study for the first time, Previous studies corrects that overexpressed PSEN1 and PSEN2 gene expression outcomes protein expression of this genes also (Williamson et al. 2009).

In conclusion, the present study demonstrated that vildagliptine may improve cellular viability against A β . When over production of A β is thought to be one of major causative factor in AD, vildagliptine may investigate with pharmacoepidemiological researches for a possible treatment approach for patients with diabetes and AD, who are already using an agent for curing hyperglycemia.

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