Pioglitazone ameliorates Aβ42 deposition in rats with diet-induced insulin resistance associated with AKT/GSK3β activation

SISI YANG1*, ZHE CHEN2*, MING CAO1, RENJIE LI3, ZHIGANG WANG4 and MUXUN ZHANG5

Departments of 1Geriatrics, 2Integrated Chinese Traditional and Western Medicine, and 3Emergency, Tongji Hospital Affiliated to The Tongji Medical College of Huazhong University of Science and Technology, Wuhan, Hubei 430030; 4Department of Pathogen Biology, College of Basic Medicine, Hubei University of Chinese Medicine, Wuhan, Hubei 430065; 5Department of Endocrinology, Tongji Hospital Affiliated to The Tongji Medical College of Huazhong University of Science and Technology, Wuhan, Hubei 430030, P.R. China

Received February 2, 2016; Accepted January 26, 2017

DOI: 10.3892/mmr.2017.6342

Abstract. Pioglitazone may have potential benefits as an alternative therapeutic treatment for patients with Alzheimer’s disease (AD), particularly in individuals that also have comorbid diabetes; however, the mechanisms of action remain unclear. The present study aimed to explore the effects of pioglitazone on amyloid β, isoform 42 (Aβ42) deposition in rats with diet-induced insulin resistance (IR). Diet-induced IR model rats were established in the presence or absence of pioglitazone. Plasma glucose and insulin levels, and cerebrospinal fluid insulin levels were measured; in addition, hippocampal tissues were collected for immunohistochemical analysis of Aβ42 expression. The levels of insulin-degrading enzyme (IDE) and peroxisome proliferator-activated receptor γ (PPARγ) mRNA and protein expression were analyzed by reverse transcription-quantitative polymerase chain reaction and western blotting, respectively. In addition, the activation of glycogen synthase kinase 3β (GSK3β) induced by phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling was detected by western blotting. Results from the present study demonstrated that pioglitazone may enhance peripheral and brain insulin sensitivity in diet-induced IR model rats.

Treatment with pioglitazone ameliorated Aβ42 deposition in the hippocampus by increasing IDE and PPARγ expression. Notably, activation of the PI3K/AKT/GSK3β pathway was also demonstrated to serve a role in pioglitazone-induced Aβ42 degradation, which was abrogated by the PPARγ antagonist GW9662. Results from the present study indicated that pioglitazone may improve insulin sensitivity and ameliorate Aβ42 accumulation in rats with diet-induced IR by regulating AKT/GSK3β activation, suggesting that pioglitazone may be a promising drug for AD treatment.

Introduction

Alzheimer’s disease (AD) is a progressive, degenerative and irreversible neurological disorder that is characterized by the formation of amyloid β (Aβ) plaques, neurofibrillary tangles, amyloid angiopathy and loss of neurons and synapses (1). Accumulating evidence indicates that obesity in middle age increases the risk for AD and there is an association between AD and glucose metabolism disorder (2). A number of epidemiological studies have also demonstrated that type 2 diabetes mellitus (T2DM) appeared to be a significant risk factor for AD (3,4). In patients with late-onset diabetes, the incidence of AD was observed to be two times higher compared with elderly patients without diabetes. Although the mechanisms leading to AD are numerous and complex, the most common factor among patients diagnosed with AD was reported to be insulin resistance (IR) (5). Another study hypothesized that peripheral IR may be able to induce nervous system damage and cognitive impairment in patients (6).

IR is the decreased sensitivity of target organs to insulin and is associated with metabolic defects and hyperinsulinemia. Notably, continuous hyperinsulinemia may impair the function of the blood-brain barrier and abrogate insulin activity (7). One study demonstrated that long-term neuronal exposure to a high-level insulin environment led to neuronal degeneration and caused irreversible cognitive dysfunction (8). In addition, long-term IR in the peripheral tissues promoted IR in the brain by suppressing insulin uptake and accelerating the accumulation of Aβ isoform 42 (Aβ42) accumulation in the brain (9). Signaling pathways associated with IR and Aβ
accumulation in the brain are complex. Insulin binding leads to autophosphorylation of the insulin receptors, which initiates several signaling cascades such as the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) phosphorylation of glycogen synthase kinase 3 (GSK3) (10). GSK3α may promote the generation of Aβ by modulating the cleavage of the amyloid precursor protein (APP) at the γ-secretase site, a site that may determine Aβ amyloidogenicity (11). A previous study demonstrated that abolishing neuronal insulin signaling in neuron-specific insulin receptor-knockout mice led to protein hyperphosphorylation and activity, one of the hallmarks of AD, which was partly due to alteration of AKT and GSK3 phosphorylation (12).

The insulin sensitizer pioglitazone is an agonist of peroxisome proliferator-activated receptor γ (PPARγ). A previous study indicated that pioglitazone improved learning ability and attenuated Aβ deposition and t pathology in AD model mice (13). Treatment with pioglitazone may also enhance cognition in patients with diabetes and AD, and decrease fasting plasma insulin levels, indicating enhanced insulin sensitivity (14). Notably, a randomized pilot clinical trial suggested that pioglitazone was a well-tolerated treatment in patients with AD (15). No serious or unanticipated adverse events or clinical laboratory changes attributable to pioglitazone were observed over long-term exposure in non-diabetic patients with AD (16). Although pioglitazone provided a potential benefit and therapeutic method for the treatment of AD, particularly in patients with comorbid diabetes, details of the functional mechanisms remain unclear.

In conclusion, results from the present study indicated that pioglitazone may improve both peripheral and brain insulin sensitivity in diet-induced IR model rats. Pioglitazone reduced Aβ42 deposition associated with AKT/GSK3β activation by increasing the levels of insulin-degrading enzyme (IDE) and PPARγ expression. These data provide additional support and emphasize the potential benefit of pioglitazone in managing glucose metabolism and ameliorating Aβ42 accumulation in patients with AD.

Materials and methods

Animals and treatment. Healthy male Wistar rats (age, 10-12 weeks; 180-220 g, n=40) were purchased from the Experimental Animal Center of Tongji Medical College (Huazhong University of Science and Technology, Wuhan, China). All animals were treated in accordance with the international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals. All of the protocols were approved by the Institutional Ethics Committee of Huazhong University of Science and Technology. They were provided with food and water ad libitum, and kept under controlled temperature (22±2°C), humidity (55±5%) and 12 h light/dark cycle. All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were randomly divided into two groups: An IR model group (n=30) and a control group (n=10). The control group was fed on a standard diet and IR model rats were fed a high glucose, high fat and high protein diet (HD; calorie percentage: 58.8% fat, 26.0% carbohydrate and 15.2% protein) from the age of 10-12 weeks and were maintained on the HD diet for ≥12 weeks. HD-induced IR model rats were detected by homeostasis model assessment-insulin resistance (HOMA-IR) using the formula: HOMA-IR=fasting insulin (mIU/l) x fasting blood glucose (mM)/22.5 (13). Body weight and whole blood glucose were measured prior to starting the defined diet, and measurements were made once per week thereafter. Rats that did not develop IR were excluded from the study, and the final number of IR model rats used in the present study was 24.

All rats with HD-induced IR were randomly divided into three subgroups; n=8 per group: i) The untreated IR model group (n=8); ii) the IR + pioglitazone group, to which pioglitazone (20 mg/kg; Merck KGaA, Darmstadt, Germany) was administered intragastrically; and iii) the IR + pioglitazone + GW9662 group, which received pioglitazone (20 mg/kg; intragastric administration) and an intraperitoneal injection of the PPARγ inhibitor GW9662 (1 mg/kg; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Pioglitazone and GW9662 were dissolved in saline, and the treatments were administered daily for 4 weeks.

Cerebrospinal fluid (CSF) collection. Rats were anesthetized with 20% ethyl carbamate (urethane; Sigma-Aldrich; Merck KGaA), and 20 μl CSF was collected from the cisterna magna of each rat and stored in Eppendorf vials containing Pefabloc SC (1.0 mM; Pentapharm Ltd., Aesch, Switzerland) at -80°C.

Glucose and insulin measurement. Blood was collected from the tail vein of each rat with a metal needle and blood glucose levels were measured using the glucose oxidation method and a OneTouch UltraLink Blood Glucose Meter (LifeScan, Inc., Milpitas, CA, USA). At the end of the experiment the rats were decapitated for arterial blood. After centrifugation at 12,000 x g for 10 min at 4°C, the plasma was collected. Plasma and CSF insulin were determined using a rat insulin kit by sandwich enzyme-linked immunosorbent assay (ELISA; Mercodia AB, Uppsala, Sweden), according to the manufacturer's protocol. All the experiments were repeated three times.

Tissue preparation. Rats were decapitated following anesthesia with 20% ethyl carbamate (urethane) (Tianjin Kemiou Chemical Reagent Co., Ltd, Tianjin, China) and the hippocampus was rapidly dissected from the hemisphere on an ice-cold board. The right hippocampus was fixed in 4% paraformaldehyde (Sigma-Aldrich) at 4°C overnight and embedded in paraffin for immunohistochemical analysis. The left hippocampus was stored at -80°C for further analysis by western blotting and reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Immunohistochemistry. Following removal of paraffin, coronal brain sections (4 μm) were sequentially treated with 0.25% Triton-X 100 in Tris-buffered saline (TBS; 50 mM Tris-HCl, 0.9% NaCl, pH 7.6) for 10 min, blocked with 5% BSA (Sigma-Aldrich; Merck KGaA) at room temperature for 60 min and incubated with primary anti-Aβ42 antibody (rabbit anti-rat; 1:500; ab32136; Abcam, Cambridge, MA, USA) at room temperature for 2 h. Following primary antibody incubation, sections were washed with TBS for 10 min, blocked with
5% normal goat serum (C0265; Beyotime, Nanjing, China) in TBS for 15 min, incubated with biotin-conjugated secondary antibody (mouse anti-rabbit; 1:500; sc-2491; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) in TBS plus 0.05% normal goat serum for 1 h, washed with TBS for 10 min and visualized with 3,3′-diaminobenzidine (Sigma-Aldrich; Merck KGaA) using the Histostain-SP IHC kit (Zymed; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Sections (five per experimental group) were evaluated under a Nikon Eclipse Ti-S microscope (Nikon Corporation, Tokyo, Japan).

**RT-qPCR.** Total RNA was extracted from left hippocampus tissues using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Equal amounts of RNA (1 μg) were used to synthesize cDNA using the Superscript First-Strand Synthesis kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol. Synthesized cDNAs were amplified using SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and a StepOne Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). β-actin was used as an internal standard to control the variability in amplification. The data were analyzed by the 2^(-ΔΔCt) method (15). The following primers were used: PPARγ, forward 5′-ACCCATTGCAGCAAAAAAC-3′ and reverse 5′-GGATCGCTTACAACCTACC-3′; IDE, forward 5′-TTTCTCTGAGCACCCTTTCCA-3′ and reverse 5′-CAGATGACGGAGATAATGACCACG-3′; APP, forward 5′-ACCCATCAGGAGCATAACCC-3′ and reverse 5′-GGATCGCTTACAACCTACC-3′; β-actin, forward 5′-TCATGAGGTATGCATCGAGG-3′ and reverse 5′-CTTCTACAATGAGCTGGTG-3′. The amplification conditions were: 5 min at 94°C, 35 cycles of 45 sec at 94°C, 1 min at 56°C and 1 min at 72°C; followed by 10 min at 72°C. All the experiments were repeated three times.

**Western blotting.** Total protein was extracted from the left hippocampus, washed once with ice-cold PBS, and lysed with a lysis buffer (50 mM Tris HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 0.2% deoxycholic acid and 1:100 protease inhibitor cocktail). Lysates were centrifuged for 10 min at 12,000 x g at 4°C and supernatants were analyzed for protein concentration using a BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Proteins were denatured at 95°C (10 min, 30 μg), separated by electrophoresis (SDS-PAGE, 4% stacking gel and 10% separating gel) and transferred onto nitrocellulose membranes (Sigma-Aldrich; Merck KGaA). Membranes were washed in TBS with 0.05% Tween-20 (TBST) prior to blocking with 5% skimmed milk at 37°C for 1 h, followed by overnight incubation at 37°C with polyclonal rabbit antibodies anti-Aβ42 (1:5,000; ab32136), anti-IDE (1:500; ab32216), anti-phosphorylated (p)-AKT Thr308 (1:500; ab194875), total AKT (1:500; ab79360; all from Abcam), PPARγ antibody (1:1,000; sc-7196), β-actin (1:1,000; sc-7210; both from Santa Cruz Biotechnology, Inc.), total GSK3β (1:1,000; 5676) or p-GSK3β Ser9 (1:1,000; 9327; both from Cell Signaling Technology, Inc., Danvers, MA, USA), followed by 1 h incubation at 37°C with secondary antibodies (horseradish peroxidase conjugated goat anti-rabbit; ab98485; 1:10,000; Abcam). Immunoreactive bands were detected by enhanced chemiluminescence peroxidase-catalyzed peroxidation of luminol in the system and were analyzed using Quantity One Software version 4.6.3 (Bio-Rad Laboratories, Inc.). To analyze the results across different experiments (n≥3), optical density levels were normalized to β-actin in the respective western blots.

**Statistical analysis.** Data were analyzed by one-way analysis of variance, followed by post hoc tests (Newman-Keuls) or Student's t-test using Prism 5 (version 5; GraphPad Software, Inc., La Jolla, CA, USA). Results are expressed as the mean ± standard deviation, and P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Pioglitazone improves insulin sensitivity in the peripheral and the central nervous system of HD-induced IR model rats.** IR model rats were established by feeding a HD diet for 12 weeks; HOMA-IR assessment identified that 24 out of 30 rats (80%) developed IR (data not shown). Rats fed a HD diet gained 20% more weight and had significantly higher HOMA-IR compared with control rats (Fig. 1A and B, respectively). Plasma insulin levels were also significantly higher in IR model rats compared with rats in the control group (Fig. 1C); however, plasma glucose levels were similar between the two groups (Fig. 1D). These results confirmed the successful establishment of IR model rats. By contrast, there was a significant decrease in the levels of insulin in the CSF in IR model rats compared with control rats (Fig. 1E), suggesting decreased insulin signaling in the brain of IR rats. Following treatment with pioglitazone (Fig. 1), HOMA-IR and plasma insulin levels were significantly reduced compared with rats in the untreated IR group, however the weight and the levels of plasma glucose and CSF insulin were not significantly changed.

**Pioglitazone reduces Aβ42 accumulation in the hippocampus in IR model rats.** To investigate whether pioglitazone is able to inhibit Aβ42 deposition in rat brains, the hippocampus of rats from each group were stained with a polyclonal antibody against Aβ42. Only background staining was observed in control rats, whereas Aβ42 immunostaining was stronger and detected in more neurons throughout the hippocampus in IR rats (Fig. 2). IR model rats treated with pioglitazone for 4 weeks exhibited significantly reduced levels of Aβ42 accumulation, particularly in the dentate gyrus of the hippocampus (reduce the levels of Aβ42 accumulation/concentration in the brain (reduce the levels of Aβ42 accumulation in the brains of IR rats).
YANG et al.: PIOGLITAZONE AMELIORATES Aβ42 DEPOSITION VIA AKT/GSK3β ACTIVATION

...and PPARγ increased (Figs. 3 and 4), and the levels of APP mRNA and Aβ42 protein decreased compared with untreated IR rats (Figs. 3 and 4, respectively). These data suggested that IDE and PPARγ may be involved in the inhibitory effect of pioglitazone on Aβ42 accumulation.

**Figure 1.** Pioglitazone treatment improves insulin sensitivity in HD-induced IR rats. Rats were assessed following 16 weeks (12 weeks to establish the IR model plus 4 weeks treatment/HD-diet) on a normal or HD diet for different indices of HD-induced IR, including: (A) Body weight, (B) HOMA-IR, (C) plasma insulin, (D) plasma glucose and (E) CSF insulin. Data are presented as the mean ± standard deviation. Control group, n=10; IR model rat groups, n=8/group. *P<0.05 and **P<0.01. CSF, cerebrospinal fluid; HD, high glucose, high fat and high protein diet; HOMA-IR, homeostasis model assessment-insulin resistance; IR, insulin resistance.

**Figure 2.** Pioglitazone treatment alleviates Aβ42 accumulation in the hippocampus of IR model rats. (A) Immunohistochemical analysis of Aβ42 in the hippocampus of the different rat groups. Scale bar, 100 μm. (B) Aβ42-positive cells were increased in IR model rats compared with control, but decreased following pioglitazone treatment. Data are presented as the mean ± standard deviation. Control group, n=10; IR model rat groups, n=8/group. *P<0.05 and ***P<0.001. Aβ42, amyloid β, isoform 42; IR, insulin resistance.

**Figure 3.** Pioglitazone treatment increases the expression of IDE and PPARγ mRNA. Reverse transcription-quantitative polymerase chain reaction was used to assay the expression of APP, IDE and PPARγ mRNA in the different experimental rat groups. Control group, n=10; IR model rat groups, n=8/group. Data are presented as the mean ± standard deviation. *P<0.05, **P<0.01 and ***P<0.001. APP, amyloid precursor protein; IDE, insulin-degrading enzyme; IR, insulin resistance; PPARγ, peroxisome proliferator-activated receptor γ.

Pioglitazone ameliorates Aβ42 accumulation via the AKT/GSK3β-signaling pathway. To investigate the molecular mechanisms by which pioglitazone reduces Aβ42 accumulation in IR model rats, the protein expression levels of p-AKT and p-GSK3β, which are two important protein kinases in the insulin-signaling pathway, were examined (Fig. 5). There was a marked increase in the phosphorylation of both AKT at Thr308 and GSK3β at Ser9 in the hippocampus of IR rats treated with pioglitazone compared with rats in the untreated IR group, whereas the total levels of expression of these two kinases (AKT and GSK3β) expression between the control and IR groups were unchanged.

As an agonist of PPARγ, pioglitazone treatment was demonstrated to result in the increased expression of IDE and PPARγ and the decreased accumulation of Aβ42 (Figs. 3 and 4). By contrast, IR + pioglitazone rats co-treated with the PPARγ antagonist GW9662 exhibited a decrease in the levels of AKT and GSK3β phosphorylation and Aβ42 deposition compared with IR rats treated with pioglitazone alone (Fig. 5). These data suggest that PPARγ may serve a crucial role in the pioglitazone-inhibited expression of Aβ42.

**Discussion**

A number of studies have indicated that the binding and activation of PPARγ by the TZD pioglitazone may be able to alleviate synaptic dysfunctions and cognitive impairment...
in AD mouse models and patients with AD associated with diabetes (17-19). Results from the present study demonstrated that pioglitazone may help improve both peripheral and brain insulin sensitivity in rats with HD-induced IR. Pioglitazone ameliorated Aβ42 deposition associated with AKT/GSK3β activation by increasing the expression of IDE and PPARγ.

Preclinical and clinical evidence suggest a pathological connection between AD and diabetes. Alterations in metabolism, inflammation and IR are characteristic features of both diseases (19,20). Increased Aβ42/Aβ40 and total τ expression were demonstrated to be exacerbated by IR, and hyperphosphorylated-τ and AD-amyloidosis models exhibited IR (21). Insulin reduced the generation and aggregation of Aβ, while IDE degraded Aβ, suggesting that IR may serve a major role in the progress of AD (22). The link between AD and IR may be associated with the role of insulin in brain metabolism and plasticity. Insulin passes through the blood-brain barrier and regulates glucose metabolism, this process affects amyloid (Aβ42), neuronal survival and neural network plasticity (23). A previous study also indicated elevated Aβ levels induced the removal of insulin receptors on the cell surface, thus enhancing IR (24). In addition, inflammatory responses, which were present in patients with obesity and T2DM, were indicated to be closely associated with the development of IR in both peripheral and central tissues (25). Therefore, abnormal brain IR may be an important and early pathological feature that significantly increases the risk of AD.

IDE is a highly conserved Zn²⁺-dependent endopeptidase that degrades insulin and regulates peripheral insulin levels (26), and is one of the key enzymes involved in Aβ degradation in the brain (27). Genetic variants in the haplotype block that span the IDE gene have been associated with higher levels of plasma Aβ42 and an increased risk of AD (28). Compared with peripheral insulin receptors, brain insulin receptors are not downregulated by high insulin levels, suggesting that IDE may act as an effector molecule that inhibits insulin signaling when insulin levels are high (29). In the brain of AD patients, insulin levels were abnormally low, decreasing the amount of insulin available to compete with Aβ42 for IDE (30); this is in contrast to the hypothesis that Aβ42 was degraded more effectively and the level of Aβ42 decreased. However, results from the present
study demonstrated an increased Aβ42 with lower IDE and PPARγ expression in IR rat brains. Consistent with this, a previous report demonstrated that IR induced by a high-fat diet was associated with reduced IDE levels and increased amyloidosis in an AD animal model (25). In the present study, treatment with pioglitazone led to an increase in the expression of IDE and PPARγ, and a reduction of Aβ42 accumulation in IR model rats. These results suggested that pioglitazone decreased brain Aβ42 deposition in rats with IR, possibly by activating IDE and PPARγ expression.

A previous study demonstrated that pioglitazone was a potential therapeutic method for the treatment of AD, especially in an animal model of AD comorbid diabetes (31). However, the mechanisms underlying these neuroprotective effects remain to be determined. One recent study involving fructose-induced IR model rats reported an improvement in cognitive functions owing to an increase in insulin sensitivity and antioxidant defense system, which corresponded with a decrease in IDE activity (32). An additional previous study demonstrated that pioglitazone-treated AD model mice exhibited a number of beneficial effects, including improvements in learning, lowered serum cholesterol, fewer Aβ and τ deposits in the hippocampus, and enhanced short- and long-term plasticity (33). In addition, pioglitazone was observed to suppress the hyperactivation of cyclin-dependent kinase 5 in the hippocampus of APP/presenilin 1 mutant mouse by reducing the protein expression levels of p35, which regulated Aβ-induced dendritic spine loss in the brain (31). An association has been identified between the activation of the AKT/GSK3β-signaling pathway and Aβ42-induced memory impairments (34). Dalesenol B (TL-2), a potent immunosuppressive agent with an unusual carbon skeleton, induced the nuclear translocation of β-catenin and enhanced its transcriptional activity through the AKT/GSK3β pathway to promote neuronal survival, which attenuates β-amylloid induced neuronal apoptosis (35). Melatonin was also demonstrated to exhibit neuroprotective effects against Aβ42-induced neurotoxicity through decreasing memory impairment, synaptic disorder, τ hyperphosphorylation and neurodegeneration via PI3K/AKT/GSK3β signaling in the Aβ42-treated mouse hippocampal cell lines (36). Consistent with previous studies (31,32), results from the present study also indicated that pioglitazone ameliorated Aβ42 accumulation by regulating AKT/GSK3β signaling in rats with HD-induced IR. However, additional studies are required to determine if other mechanisms are involved or affected by pioglitazone treatment.

In conclusion, results from the present study indicated that pioglitazone may improve insulin sensitivity and ameliorate Aβ42 deposition in the hippocampus of IR model rats and is associated with activation of AKT/GSK3β signaling via increasing the expression of IDE and PPARγ. These results provide supporting evidence and emphasize a potential benefit of pioglitazone in the management of glucose metabolism and the treatment of AD.

Acknowledgements

The present study was supported by a grant from the National Natural Science Foundation of China (grant no. 81503426).

References

1. Musiek ES and Holtzman DM: Three dimensions of the amyloid hypothesis: Time, space and ‘wingmen’. Nat Neurosci 18: 800-806, 2015.
2. Talbot K and Wang HY: The nature, significance, and glutamin-like-peptide-1 analog treatment of brain insulin resistance in Alzheimer’s disease. Alzheimers Dement 10 (1 Suppl): S12-S25, 2014.
3. Tolppanen AM, Solomon A, Soininen H and Kivipelto M: Midlife vascular risk factors and Alzheimer’s disease: Evidence from epidemiological studies. J Alzheimers Dis 32: 531-540, 2012.
4. Toda N, Ayajiki K and Okamura T: Obesity-induced cerebral hypoperfusion derived from endothelial dysfunction: One of the risk factors for Alzheimer’s disease. Curr Alzheimer Res 11: 733-744, 2014.
5. Craft S: Insulin resistance syndrome and Alzheimer disease: Pathophysiologic mechanisms and therapeutic implications. Alzheimer Dis Assoc Disord 20: 298-301, 2006.
6. Wang F, Song YF, Yin J, Liu ZH, Mo XD, Wang DG, Gao LP and Jing YH: Spatial memory impairment is associated with hippocampal insulin signals in ovariec tomized rats. PLoS One 9: e104450, 2014.
7. Tucek Z, Toth P, Sosnowska D, Gautam T, Mitschelen M, Koller A, Szalai G, Sonntag WE, Ungvari Z and Csiszar A: Obesity in aging exacerbates blood-brain barrier disruption, neuroinflammation, and oxidative stress in the mouse hippocampus: Effects on expression of genes involved in beta-amyloid generation and Alzheimers disease. J Gerontol A Biol Sci Med Sci 69: 1212-1226, 2014.
8. Grasso G, Mielczarek P, Niezioleka M and Stibbe J: Metabolism of cryptic peptides derived from neuropeptide FF precursors: The involvement of insulin-degrading enzyme. Int J Mol Sci 15: 16787-16799, 2014.
9. Park SA: A common pathogenic mechanism linking type-2 diabetes and Alzheimer’s disease: Evidence from animal models. J Clin Neurol 7: 10-18, 2011.
10. Baker LD, Cross DJ, Minoshima S, Watson GS and Craft S: Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. Arch Neurol 68: 51-57, 2011.
11. Jolivalt CG, Lee CA, Beiswenger KK, Smith JL, Orlov M, Torrance MA and Masliah E: Defective insulin signaling pathway and increased glycogen synthase kinase-3 activity in the brain of diabetic mice: Parallels with Alzheimer’s disease and correction with exenatide. J Biol Chem 283: 42496-42506, 2008.
12. Metzger B, Gautham D, Surjo D, Uski K, Baudler S, Schubert D, Kondo T, Alber J, Galldiks N, Küstermann E, et al: Role for neuronal insulin resistance in neurodegenerative diseases. Proc Natl Acad Sci USA 101: 3000-3005, 2005.
13. Sato T, Hanyu H, Hirao K, Kanetaka H, Sakurai H and Inawato T: Efficacy of PPAR-γ agonist pioglitazone in mild Alzheimer disease. Neurobiol Aging 32: 1626-1633, 2011.
14. Nicolakakis N, Aboutkassim T, ფngali B, Leceux C, Fernandes P, Rosa-Neto P, Tong XK and Hamel E: Complete rescue of cerebrovascular function in aged Alzheimer’s disease transgenic mice by antioxidant and pioglitazone, a peroxisome proliferator-activated receptor γ agonist. J Neurosci 28: 9287-9996, 2008.
15. Geldmacher DS, Fritsch T, McClendon MJ and Landreth G: A randomized pilot clinical trial of the safety of pioglitazone in treatment of patients with Alzheimer’s disease. Arch Neurol 68: 85-50, 2011.
16. Wang Z, Yan Z, Zhang B, Rao Z, Zhang Y, Liu J, Yu L, Zhao Y, Yang B, Wu T and Gao J: Identification of a 5-gene signature for clinical and prognostic prediction in gastric cancer patients upon microarray data. Med Oncol 30: 678, 2013.
17. Pérez MJ and Quintanilla RA: Therapeutic actions of the thiazolidinediones in Alzheimer’s disease. PPAR Res 2015: 957248, 2015.
18. Craft S: Insulin resistance syndrome and Alzheimer’s disease: Age- and obesity-related effects on memory, amyloid, and inflammation. Neurobiol Aging 26: 65-69, 2005.
19. Sato N, Takeda S, Uchio-Yamada K, Ueda H, Fujisawa T, Rakugi H and Morishita R: Role of insulin signaling in the interaction between Alzheimer disease and diabetes mellitus: A missing link to therapeutic potential. Curr Aging Sci 4: 118-127, 2011.
20. Najem D, Bamji-Mirza M, Chang N, Liu QY and Zhang W: Insulin resistance, neuroinflammation, and Alzheimer's disease. Rev Neurolsci 25: 509-525, 2014.

21. Mayeux R, Hong LS, Tang MX, Manly J, Stern Y, Schuff N and Mehta PD: Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: Relation to age, mortality, and risk. Neurology 61: 1185-1190, 2003.

22. Mayeux R, Honig LS, Tang MX, Manly J, Stern Y, Schupf N and Mehta PD: Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: Relation to age, mortality, and risk. Neurology 61: 1185-1190, 2003.

23. Qiu WQ and Folstein MF: Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: Review and hypothesis. Neurobiol Aging 27: 190-198, 2006.

24. Neumann KF, Rojo L, Navarrete LP, Farías G, Reyes P and Maccioni RB: Insulin resistance and Alzheimer's disease: Molecular links & clinical implications. Curr Alzheimer Res 5: 438-447, 2008.

25. Zhao WQ, De Felice FG, Fernandez S, Chen H, Lambert MP, Quon MJ, Krafft GA and Klein WL: Amyloid beta oligomers induce impairment of neuronal insulin receptors. Faseb J 22: 246-260, 2008.

26. Ho L, Qin W, Pompl PN, Xiang Z, Wang J, Zhao Z, Peng Y, Cambareri G, Rocher A, Mobbs CV, et al: Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. FASEB J 18: 902-904, 2004.

27. Fernández-Gamba A, Leal MC, Morelli L and Castaño EM: Insulin-degrading enzyme structure-function relationship and its possible roles in health and disease. Curr Pharm Des 15: 3644-3655, 2009.

28. Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE, Selkoe DJ and Guenette S: Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc Natl Acad Sci USA 100: 4162-4167, 2003.

29. Zahniser NR, Goens MB, Hanaway PJ and Vinyard JV: Characterization and regulation of insulin receptors in rat brain. J Neurochem 42: 1354-1362, 1984.

30. Cook DG, Leverenz JB, McMillan PJ, Kulstad JJ, Erickson S, Roth RA, Schellenberg GD, Jin LW, Kovacina KS and Craft S: Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon 4 allele. Am J Pathol 162: 313-319, 2003.

31. Chen J, Li S, Sun W and Li J: Anti-diabetes drug pioglitazone ameliorates synaptic defects in AD transgenic mice by inhibiting cyclin-dependent kinase 5 activity. PLoS One 10: e0123864, 2015.

32. Yin QQ, Pei JJ, Xu S, Luo DZ, Dong SQ, Sun MH, You L, Sun ZJ and Liu XP: Pioglitazone improves cognitive function via increasing insulin sensitivity and strengthening antioxidant defense system in fructose-drinking insulin resistance rats. PLoS One 8: e59313, 2013.

33. Searcy JL, Phelps JT, Pancani T, Kadish I, Popovic J, Anderson KL, Beckett TL, Murphy MP, Chen KC, Blalock EM, et al: Long-term pioglitazone treatment improves learning and attenuates pathological markers in a mouse model of Alzheimer's disease. J Alzheimers Dis 30: 943-961, 2012.

34. Lee HK, Kumar P, Fu Q, Rosen KM and Querfurth HW: The insulin/Akt signaling pathway is targeted by intracellular beta-amyloid. Mol Biol Cell 20: 1533-1544, 2009.

35. Zhu X, Wang S, Yu L, Yang H, Tan R, Yin K, Jin J, Zhao H, Guan D and Xu Y: TL-2 attenuates β-amyloid induced neuronal apoptosis through the Akt/GSK-3β/β-catenin pathway. Int J Neuropsychopharmacol 17: 1511-1519, 2014.

36. Kwon KJ, Kim HJ, Shin CY and Han SH: Melatonin potentiates the neuroprotective properties of resveratrol against beta-amyloid-induced neurodegeneration by modulating AMP-activated protein kinase pathways. J Clin Neurol 6: 127-137, 2010.