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Short Communication

Geniposide decreases the level of $A\beta_{1-42}$ in the hippocampus of streptozotocin-induced diabetic rats

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Although cognitive dysfunction in diabetic patients has been explored extensively, diabetic complications of the central nervous system have not been studied. We have reported previously that geniposide has neurotrophic and neuroprotective activities with the activation of glucagons-like peptide 1 receptor, and regulates glucose-stimulated insulin secretion *in vitro*. But the role of geniposide on diabetic complications, especially on the neurodegenerative diseases, remains to be investigated. In this study, we investigated the effect of geniposide on the level of $A\beta_{1-42}$ in the hippocampi of streptozotocin-induced diabetic rats and explored its possible mechanism. The results demonstrated that, accompanied with the improvement of insulin and blood glucose, treatment with geniposide decreased the $A\beta_{1-42}$ level and improved the expression of insulin-degrading enzyme, which is the key degrading enzyme of Aβ peptide. The results of present study will help to understand the biochemical mechanisms of neuronal dysfunction and death in diabetes and to develop an efficient therapeutic strategy on Alzheimer's disease.

Keywords alzheimer's disease (ad); β-amyloid (aβ); diabetes; geniposide; insulin-degrading enzyme (ide)

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Introduction

Although there is still debate whether there is a causative association between diabetes mellitus (DM) and Alzheimer's disease (AD), the cognitive dysfunction and dementia have recently been proven to be the common complications of DM [1,2]. Since the initial Rotterdam study suggested an increased risk of dementia and AD in patients with type 2 diabetes mellitus (T2DM), a number of clinical and epidemiological studies have further provided direct evidence of a link between diabetes and AD [2–4]. Furthermore, several studies have demonstrated that phenotypes associated with obesity and/or insulin resistance have an increased risk of cognitive decline and dementia, including not only vascular dementia, but also AD [5,6]. However, the mechanisms through which diabetes impact AD are not well understood.

Accumulating evidence has demonstrated that hyperglycemia, vascular disease, and insulin resistance may play important roles in the pathophysiology of cognitive dysfunction and cerebral lesions in diabetes [7]. Alterations in insulin function and glucose homoeostasis in the periphery tissues may affect brain insulin and its receptor functions, promoting the production of β-amyloid and inducing tau phosphorylation [8,9]. All these results suggested that regulation on blood glucose and insulin in diabetes might be beneficial for inhibiting the development of AD.

We reported previously that geniposide, one of the main iridoid glycosides of *Gardenia* fruit, showed neurotrophic and neuroprotective activities through the activation of glucagons-like peptide 1 receptor (GLP-1R) [10–12]. Additionally, pre-incubation with geniposide prevented $A\beta_{1-42}$-induced cell injury in primary cultured cortical neurons, and geniposide also induced the expression of insulin-degrading enzyme (IDE), a major protease of Aβ, in a dose-dependent manner. Furthermore, inhibition of IDE and RNAi on *Glp1r* gene decreased the neuroprotecion of geniposide in $A\beta_{1-42}$-treated cortical neurons [13]. We hypothesized that geniposide might play an essential role in the diabetic complications, especially for neurodegenerative diseases, such as AD.

To investigate the role of geniposide and its possible mechanisms in diabetic complications, we determined the levels of $A\beta_{1-42}$ and IDE in streptozotocin (STZ)-induced diabetic rats. The results demonstrated that, accompanied with the up-regulation on the expression of IDE, geniposide significantly decreased the level of $A\beta_{1-42}$ in the brain of diabetic rats.

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Materials and Methods

Materials

Materials were obtained from the following sources: Geniposide (UR20060421, purity>99.5%) from Sichuan Dicotyledonous Bio-tech (Chengdu, China); metformin hydrochloride from Sigma (St Louis, USA); $A\beta_{1-42}$ enzyme-linked immunosorbent assay (ELISA) kit from Wako (Shizuoka, Japan); total cholesterol and triglyceride from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China); polyvinylidene difluoride (PVDF) membranes and enhanced chemoluminescence (ECL) western blot kit from Millipore (Darmstadt, Germany), antibodies against rat insulin-degrading enzyme and β-actin from Cell Signaling Technology (Danvers, USA).

Cell culture and treatment

Human neuroblastoma SH-SY5Y cells were purchased from the Cell Bank of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China), and routinely cultured in a mixture media of Dulbecco's modified Eagle's medium and Ham's F12 supplemented with 10% fetal bovine serum. Cells were harvested when they reached 80%–90% confluency and then seeded at a density of 5×10^5 cells/ml into wells of six-well plates. The plates were incubated overnight in 5% CO_2 at 37°C. Different concentrations of geniposide or equal volume of phosphate-buffered saline (control) were added into the wells and the plates were incubated for another 24 h. After removal of the media, cells in each well were lyzed in RIPA and equal amounts of cell lysates were subject to western blot analysis to determine the level of IDE protein.

Induction of diabetes

Male Sprague–Dawley rats, aged 6–8 weeks, were housed in cages (two rats per cage) on a 12-h light/dark cycle at an ambient temperature of 20–25°C. The rats had free access to water and diet. Animal experiments described in the present study were performed in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals. All the experimental protocols were also approved by the Institutional Animal Care and Use Committee at Chongqing Technology and Business University. The rats were subjected to overnight fasting (deprived of food for 16 h, but with free access to water) and intraperitoneal injection with STZ (30 mg/kg body weight) dissolved in 0.1 M sodium citrate buffer (pH 4.5) or vehicle (0.1 M sodium citrate buffer only). Three hours after the STZ injection, the rats were allowed to eat freely again. After a week, blood was collected from the tail vein and analyzed for blood glucose using a glucometer (Qiangsheng, Shanghai, China). Animals showing fasting blood glucose >11.1 mM were considered diabetic and used for further experiments.

Geniposide treatment and biochemical analysis

To evaluate the role of geniposide on blood glucose, geniposide treatment with intragastric administration was started on the 8th day after STZ injection and continued for 46 days (metformin was used as the positive control). At the end of the treatment, rats were sacrificed by cervical decapitation after being anesthetized and blood was collected. Blood glucose, TC, and triglyceride were determined by biochemical methods. Blood insulin and $A\beta_{1-42}$ were determined by using ELISA kits according to manufacture's instruction.

Western blot analysis

An aliquot of 20 μg of each sample was separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis at room temperature and transferred on PVDF membrane for 1 h at 4°C. Membranes were blocked in a 5% fat-free milk solution in TBST (Tris buffer containing 0.5% Tween-20), then probed with goat anti-IDE polyclonal antibody (1 : 2000; Santa Cruz, Santa Cruz, USA) and then incubated with horseradish peroxide-conjugated anti-goat IgG antibody (1 : 5000, Santa Cruz). Immunoreactive signals were detected with an ECL Advance Western Blotting Detection Kit (Amersham Bioscience, Piscataway, USA). Densitometric analysis was performed by using the software of Quantity One (Bio-Rad, Murich, Germany).

Statistical analysis

Data were presented as mean ± SD. Analysis of variance was carried out by using Origin software (Bio-Rad, USA), with $P < 0.05$ considered statistically significant, as assessed by Student's *t*-test with corrections for multiple comparisons to a single group (Dunnett's *t*-test) and between multiple groups (Bonferroni and Tukey's tests).

Results

Geniposide modulates insulin, blood glucose, total cholesterol, and triglyceride

As shown in **Table 1**, oral administration of metformin (800 mg/kg/day) for 46 days significantly decreased blood glucose level and increased insulin level. When compared with the control group, treatment with 25 mg/kg geniposide decreased blood glucose level ($P < 0.05$) and increased the level of blood insulin in STZ-induced diabetic rats. Furthermore, geniposide ameliorated the symptoms of hyperlipidemia by decreasing the levels of total cholesterol ($P < 0.05$) and triglyceride ($P < 0.01$), but metformin had no significant effects.

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Table 1 Effects of geniposide on blood glucose, insulin, total cholesterol (TC), and triglyceride (TG) in STZ-induced diabetic rats

Groups	Dose (mg/kg)	Glu (mmol/l)		Ins (μU/ml)	TC (mmol/l)	TG (mmol/l)
		Previous	After 46 days			
Normal	/	6.16 ± 0.58	6.65 ± 0.71	9.68 ± 3.51	1.27 ± 0.16	1.26 ± 0.37
Model	/	15.19 ± 3.88	20.35 ± 7.91	3.29 ± 0.84	1.75 ± 0.23	1.37 ± 0.52
Geniposide	12.5	15.39 ± 3.51	13.01 ± 4.33	3.67 ± 1.20	1.58 ± 0.45	0.94 ± 0.29*
Geniposide	25	15.89 ± 3.71	9.98 ± 5.35*	4.48 ± 1.96	1.40 ± 0.24**	0.91 ± 0.32*
Metformin HCl	800	15.23 ± 3.46	7.64 ± 3.55**	6.21 ± 4.16	1.62 ± 0.34	1.93 ± 0.76

Data were expressed as the mean ± SD.
Glu, glucose; Ins, insulin.
*$P < 0.05$.
**$P < 0.01$ vs. STZ-induced diabetic group. $n = 9$.

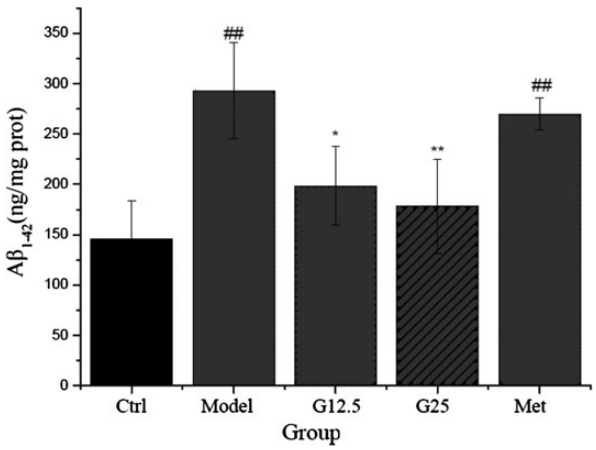

Figure 1 Geniposide decreased the level of $A\beta_{1-42}$ in the hippocampus of STZ-induced diabetic rats After the rats were sacrificed by cervical decapitation, the hippocampal tissues were stripped and homogenated and the total protein were determined by Lowry method. $A\beta_{1-42}$ level was quantified by using commercial ELISA kits according the instructions from the supplier. $^{\#\#}P < 0.01$ vs. normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. model group. $G_{12.5}$, G_{25}, and Met represented the groups treated with 12.5 mg/kg geniposide, 25 mg/kg geniposide, and 800 mg/kg metformin HCl, respectively. $n = 9$.

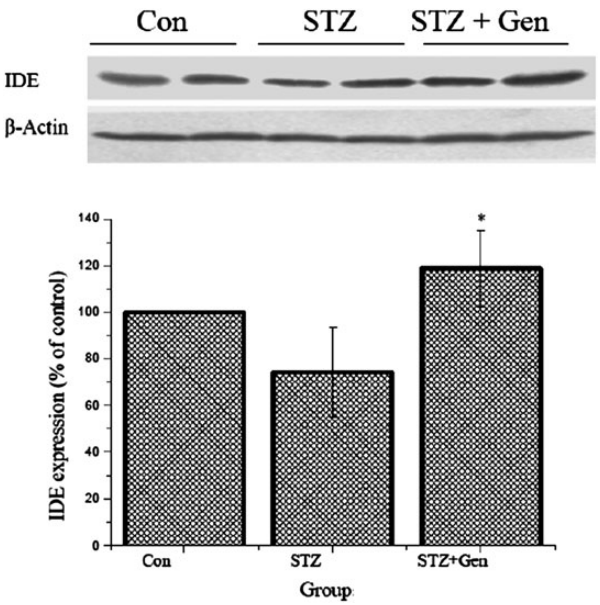

Figure 2 Geniposide improved the expression of IDE in the hippocampus of STZ-induced diabetic rats Con: normal group; STZ: model group; STZ + Gen: 12.5 mg/kg geniposide-treated group. Experiments were performed in triplicate. $^{\#}P < 0.05$ vs. STZ-induced model group. $n = 6$.

Geniposide decreases $A\beta_{1-42}$ content

In the present study, we observed that the content of $A\beta_{1-42}$ in the hippocampus of STZ-induced diabetic rats was much higher than that of the control group ($P < 0.01$), but geniposide markedly decreased the level of $A\beta_{1-42}$ in the hippocampus of diabetic rats. Treatment with 12.5 and 25 mg/kg geniposide for 46 days decreased the content of $A\beta_{1-42}$ from 293.1 ± 47.7 to 198.6 ± 19.2 and 178.1 ± 46.6 ng/mg protein, respectively (**Fig. 1**). However, metformin, a widely used antihyperglycemic agent, had no distinctive effect on the production of $A\beta_{1-42}$.

Geniposide enhances IDE expression

To investigate the biochemical mechanisms by which geniposide regulates $A\beta_{1-42}$ in diabetic animals, we measured the expression of IDE, a key proteolysis enzyme of Aβ peptide, in the hippocampus of experimental rats. The results demonstrated that administration with 12.5 mg/kg geniposide markedly reversed the effect of STZ on the expression of IDE in the hippocampi of diabetic rats (**Fig. 2**).

We further used neuroblastoma SH-SY5Y cells to confirm the role of geniposide on IDE expression in neurons. After incubation with different concentration of geniposide for 24 h, the level of IDE protein increased significantly in a dose-dependent manner. **Figure 3** showed that treatment with 10 μM geniposide increased the protein level ~2.5-fold, when compared with control.

Discussion

In the present study, we showed that geniposide decreased the blood glucose level and improved the level of insulin in

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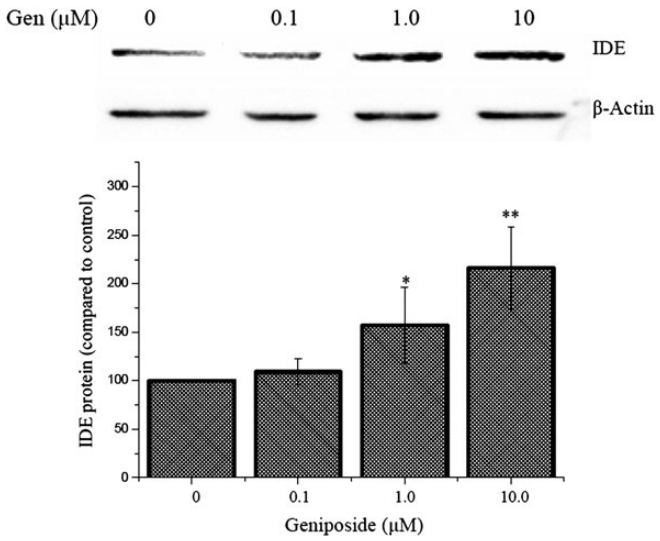

Figure 3 Geniposide increased the expression of IDE protein in a concentration-dependent manner in SH-SY5Y cells After treatment with geniposide at indicated doses for 24 h, the level of IDE protein were analyzed by western blot analysis. $*P < 0.05$ and $**P < 0.01$ vs. control group.

STZ-induced diabetic rats. Geniposide also regulated the total cholesterol ($P < 0.05$) and triglyceride ($P < 0.01$) in the periphery of diabetic animals, suggesting that geniposide might be beneficial for the treatment of hypertriglycemia in diabetes. Furthermore, it was found that, compared with the normal control animals, the level of $A\beta_{1-42}$ in the hippocampus of STZ-induced diabetic rats was increased distinctively, and geniposide could significantly decrease the level of $A\beta_{1-42}$ in hippocampus of STZ-induced diabetic rats. Although the molecular mechanisms of AD development remains unclear, there is considerable evidence supporting the so-called 'amyloid cascade hypothesis' [14,15]. Therefore, the results of the present study are helpful to understand the biochemical mechanisms of neuronal dysfunction and death in AD developed from diabetes.

Diabetes could increase the risk of AD through several biologically plausible pathways, but the relationship between diabetes and AD and the development of AD remains uncertain [16]. An important link between diabetes and AD may be related to the IDE, which is a metalloprotease enzyme responsible for insulin degradation and is also the main enzyme responsible for Aβ degradation [17,18]. IDE level has been reported to be decreased in the brains of AD patients, especially in the hippocampus [19,20]. In the present study, we showed that IDE expression in the hippocampus is reduced in the STZ-induced diabetic rats, which could be reversed by geniposide. We hypothesized that injection with STZ effectively sequesters IDE expression, reduces Aβ peptide degradation, which would increase the levels of Aβ peptides and promote many of the pathological features associated with AD, and that treatment with geniposide ameliorates this phenomenon in STZ-induced rats. Further *in vitro* and *in vivo* studies are needed to clarify the effect of geniposide on AD.

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