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Artemisinin Ameliorates Diabetic Cognitive Impairment by Improving Synaptic Plasticity *via* PI3K/Akt Pathway in Mice*

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Abstract **Objective** The present study was to clarify the improving effect of artemisinin on diabetes-induced cognitive deficit and the underlying mechanisms in mice. **Methods** Type 2 diabetes mellitus (T2DM) mouse models were established by a single dose of STZ injection (100 mg/kg, *i.p.*). Those animals were then treated with vehicle or artemisinin (40 mg/kg, *i.p.*) once daily for 4 weeks. Cognitive performances of the mice were evaluated by novel object recognition, Y maze test and Morris water maze test. After behavioral tests, the expressions of PI3K, Akt, SYN and PSD-95 proteins in the hippocampus were measured by Western blot. Changes in the synaptic ultrastructure of the hippocampal CA1 region were observed by transmission electron microscope. **Results** Our results indicated that artemisinin significantly ameliorated cognitive deficit in T2DM mice. Furthermore, PI3K and phosphorylated Akt protein levels in the hippocampus of T2DM mice treated with artemisinin were elevated, accompanied with increases in the number of hippocampal neurons, as well as the protein contents of SYN and PSD-95. Meanwhile, synaptic plasticity was also rescued, indicated by an increase in synapse number and synaptic curvature, the thickness of postsynaptic density, and a decrease in the width of synaptic cleft in the hippocampal CA1 region. **Conclusion** Taken together, these results demonstrate that artemisinin can protect T2DM mice against cognitive decline, at least partially through activating PI3K/Akt pathway to improve synaptic plasticity in the hippocampus. These findings demonstrate that artemisinin may serve as a novel therapeutic agent for diabetic cognitive impairment.

Key words diabetic cognitive deficit, synaptic plasticity, hippocampus, artemisinin, PI3K
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Clinical and epidemiological data have been confirmed that patients with type 2 diabetes mellitus (T2DM) are more susceptible to cognitive deficit leading even dementia[1-2]. Given high prevalence of diabetes, this metabolic disease-induced cognitive decline is expected to aggravate substantially and poses serious challenges to public health. However, the mechanisms underlying diabetic cognitive decline remain unclear, and the clinical treatment effect of this complication is poor. Since hippocampal neuron damage and synaptic loss contribute to cognitive deficit, reversing those abnormalities in the hippocampus can be beneficial in the improvement of cognitive performance in diabetes[3].

Increasing evidences indicate that the inactivation of the phosphoinositide 3-kinase/protein

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kinase B (PI3K/Akt) pathway contributes to the pathogenesis of diabetic cognitive deficit[4-5]. In this signaling, serine/threonine kinase Akt, a down-stream target of PI3K, which is closely related with various cellular functions, such as cell metabolism, proliferation, and differentiation[6]. Recently, studies have demonstrated that PI3K/Akt signaling in the brain plays predominant roles in neurogenesis and neuron injury, dendritic growth, and spine maturation[5, 7-8]. Furthermore, this pathway is also implicated in regulating hippocampal synaptic plasticity and cognitive performances[9]. Additionally, its activation of PI3K/Akt pathway leads to an increase in the expression of synaptophysin (SYN) and postsynaptic density-95 protein (PSD-95) [10]. Indeed, up-regulation of SYN and PSD-95 in hippocampus promotes the long-term synaptic plasticity and cognitive ability[11]. Therefore, targeting this pathway to restore hippocampal synaptic plasticity may be a therapeutic strategy for attenuating diabetic cognitive impairment.

Artemisinin (Art) is a sesquiterpene lactone compound with peroxide group[12]. Apart from its powerful anti-malaria effect, Art possesses many other pharmacological roles, including anti-inflammation, anti-oxidation, and anti-tumor[13-14]. Recent studies have shown that Art can enhance insulin sensitivity, and ameliorate various diabetic complications, such as diabetic kidney and cardiovascular disease, and diabetic retinopathy[15-16]. Moreover, this agent has been shown to have neuroprotective properties, and to alleviate lipopolysaccharide (LPS)-induced cognitive decline in mice[17-18]. Although there is no sufficient direct evidence to prove the protective effect of Art on diabetic cognitive function, these data imply that this compound and its derivatives may ameliorate diabetes-associated cognitive deficit.

In the present study, we hypothesized that Art can restore the activation of PI3K/Akt signaling in the hippocampal CA1 area of T2DM mice, and such a restoration promotes hippocampal neuron survival and synaptic plasticity, thus ameliorating cognitive deficits. To clarify the neuroprotective role of Art and investigate its mechanisms underlying diabetic cognitive decline, we first confirmed whether Art can improve cognitive function and attenuate hippocampal neuron loss in T2DM mice. We then investigated the effects of Art on the insulin signaling PI3K/Akt pathway and synaptic plasticity in the hippocampus of T2DM mice. The results suggest that Art ameliorates T2DM-associated cognitive decline maybe through activating hippocampal PI3K/Akt pathway to improve hippocampal synaptic plasticity.

1 Materials and methods

1.1 Materials

Art was purchased from Shanghai Macklin Biochemical Co., Ltd (China). Streptozotocin (STZ) and Tris were obtained from Sigma-Aldrich (USA). Anti-Akt and anti-phosphorylated Akt (p-Akt, Ser 473) antibodies were brought from Cell Signaling Biotechnology (Danvers, USA). Antibody against SYN, PSD95, β-actin, and PI3K were from Proteintech Group (Chicago, USA).

1.2 Animals

Forty male C57BL/6J mice (8 weeks, (19.0±1) g) were acquired from Changsha Tianqin Biotechnology Co., Ltd (Changsha, China). The mice were housed 4/cage with free access to food and water, and were lived in a controlled environment (humidity 60%−65%, temperature (25±1)°C) with a 12-hour light/dark cycle (lights on at 7:00 am). The animals were accessed to food and water freely. All animal protocols in this experiment were approved by the Animal Experimentation Ethics Committee of the University of South China (Permit Number: XYXK20150001) and strictly accorded with the guidelines launched by China Council on Animal Care.

1.3 Drug treatments and experimental schedule

T2DM models were established in C57BL/6J mice by a high-fat diet (HFD) feeding together with a single low dose of STZ treatment as described previously[19]. In brief, after one week of adaptation, 40 C57BL/6J mice were randomly divided into 3 groups: (1) control group (n=10); (2) T2DM group (n=15); (3) T2DM+Art group (n=15). Control group mice were fed on a chow diet throughout the experiment, while the other two groups mice were fed on HFD. In the fourth week, mice of the control group were injected with equal volume of citrate buffer solution, and the remainders were injected with a single dose of STZ (100 mg/kg, *i.p.*) dissolved in a citric acid buffer to induce T2DM. Blood was collected from the caudal vein and fasting blood glucose levels were measured in 72 h after STZ injection by enzymatic glucose oxidase peroxidase diagnostic kits. The mice were

diagnosed as T2DM if their fasting blood glucose levels were above 11.1 mmol/L. After 2 weeks STZ treatment, no dead mice were observed, and 13 mice in T2DM group and 12 mice in T2DM+Art group exhibited features of T2DM including weight loss, hyperglycemia, and hyperinsulinemia. At the end of week 6, Art (40 mg/kg, *i. p.*) was given by intraperitoneal injection once daily for 4 weeks consecutively. The selected dosage of Art were based on previous studies[18, 20] and our preliminary experiments. The mice in both T2DM group and the control group were injected with equal volume of phosphate buffer saline (PBS).

1.4 Measurement of fasting serum insulin level and insulin resistance index

After 4-week treatment with Art, the mice were anesthetized (4% chloral hydrate solution, 0.01 ml/g) and sacrificed, and then serum samples were prepared by centrifugation. Subsequently, the samples were taken to measure insulin content by using kits (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China). Insulin resistance index was calculated by the formula: IRI=fasting blood glucose (FBG)×fasting serum insulin (INS)/22.5.

1.5 Behavioral tests

1.5.1 Novel object recognition test

The novel object recognition (NOR) test was conducted as we previously described with some modifications[21]. Briefly, this test was performed in an experimental box (50 cm×30 cm×80 cm). Each mouse was acclimated for 5 min daily to explore the empty box for 2 consecutive days. On the third day, two identical objects were placed in the box, and each mouse was presented with those objects for 5 min. An hour later, the mouse was put back into the experimental box with a familiar object and a novel object for 5 min. Object exploration was considered when the distance from mouse's nose to object was less than 1 cm. In this test, the exploration track of each mouse was recorded by a video-assisted tracking system. The memory performance was identified as discrimination index= (time spent in novel object exploration−time spent in familiar object exploration)/ total exploration time×100%.

1.5.2 Y maze test

The Y maze test was used to measure hippocampus-associated spatial memory. The maze apparatus is composed of 3 arms (45 cm×14 cm×15 cm) at a 120° angle to each other, which was named A, B and C, respectively. The mice were allowed to freely explore the apparatus during a 10 min adaption period. Subsequently, each mouse was individually placed in the central point of the apparatus and freely explored for 5 min. It was considered to enter the arm successfully when its 4 paws were located in the arm. The total number of arm entry and the sequence of arm entry were recorded during the test session. The spatial memory ability was identified by index of the spontaneous alternation. The percentage of alternation=number of the sequential triplets entrance (A-B-C, A-C-B, B-A-C, B-C-A, C-A-B and C-B-A) /number of the total entrance.

1.5.3 Morris water maze test

Cognitive function of the mice was evaluated by the Morris water maze (MWM) test as we previously described[22]. Briefly, the maze apparatus consists of a circular water pool divided into 4 equal quadrants (labeled *1*, *2*, *3* and *4*) and an escape platform. The water in the pool was tepid ((25±1)° C) and colored with milk powder. On the first day, each mouse was trained to arrive at the visible escape platform, which is a black circular platform (12 cm diameter) 1 cm above the pool water. In this test, each mouse was placed in the quadrant where the platform was not located. In the next 3 consecutive days, the hidden escape platform (12 cm diameter, 2 cm below the water surface) test was carried out to determine spatial learning and memory ability of the mice. In those tests, the mice were given 3 trials (90 s/trial) per day in different quadrants. The interval between two trials was 10 min. The mice were guided to the hidden escape platform and allowed to stay there for 15 s for their failure to escape within 90 s. For all training trials, time spent (escape latency) and distance to find the platform (path length) were recorded, respectively. In the fifth day, the probe trial was performed to assess memory performance, during which the platform was removed from the water pool, and the mice were permitted to swim freely to find target quadrant for 90 s. Time taken in the target quadrant and the number across the original platform were recorded. Those data then normalized by MT-200 Morris image motion system purchased from Chengdu Technology and Market Corp (Chengdu, China).

1.6 Hematoxylin and eosin staining

The mice were euthanized by 4% chloral hydrate solution, 0.01 ml/g and then transcardial perfusion with ice-cold PBS was performed, followed by fixation with 4% paraformaldehyde for 15 min. Subsequently, whole brain was quickly harvested and immersed in 4% paraformaldehyde at room temperature for 24 h, and then stored in the 4% paraformaldehyde solution containing 30% sucrose. After fixation and dehydration, the brain specimens were bedded with paraffins, and then cut into consecutive coronal sections (4 μm). The sections were collected and stained with hematoxylin and eosin (H&E) staining. The alternation of cell morphology in the hippocampal CA1 area in 3 visual fields of each section was observed by an optical microscope (Olympus Optical; Olympus Corporation). Pathological evaluation hippocampal CA1 region was performed as we previously described[22].

1.7 Western blot

The total protein extraction from the hippocampus of mice was we previously described. In brief, the hippocampus was lysed in radio-immunoprecipitation assay (RIPA) buffer with protease and phosphatase inhibitors to extract proteins. Protein concentration of the samples was detected by using a bicinchoninic acid (BCA) protein assay kit (Beyotime, China). Then equal amount of the boiled samples (20 μg) were separated by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride membranes (Milipore, USA). After blocking with 5% bovine serum albumin for 1 h, the membranes were then incubated with primary antibodies against PI3K (1∶2 000), Akt (1∶1 000), p-Akt (1∶1 000), SYN (1∶1 000), PSD-95 (1∶1 000) and β-actin (1∶2 000), respectively, at 4℃ overnight. After being washed, the membranes were incubated with second antibodies conjugated with horseradish peroxidase (1∶6 000) for 90 min at room temperature. The protein bands were visualized by an enhanced chemiluminescence detection system, and the intensities of the bands were measured with the Bio-Rad software.

1.8 Observation of the synaptic ultrastructure by transmission electron microscope

The hippocampal CA1 region was sampled as we previously described. The samples were cut into 1 mm^3 size species and immediately fixed in a cold neutral PBS containing 2.5% glutaraldehyde for 6 h. Subsequently, the specimens were postfixed with a mixture of 1% OSO_4 and PBS for 3 h. After dehydration, the samples were embed with Epon resin and then consecutive ultra-thin sections (50 nm) were performed. Finally, sections were stained with lead citrate and uranyl acetate, and the changes in the synaptic ultrastructures of hippocampal region were observed under a transmission electron microscope (TEM, JEM-1200EX; JEOL, Tokyo, Japan). Assessment of the synaptic ultrastructures including synapse density, synaptic curvature, and the width of synaptic cleft was done as previously addressed[23].

1.9 Statistical analysis

The results were analyzed by the GraphPad Prism 9 software and the data were expressed as mean±SEM. MWM data were analyzed by using two-way ANOVA repetitive measure, followed by post hoc Bonferroni's test. The other data of this study were analyzed by Student's *t*-test (for analyzing differences between two groups) or one-way analysis of variance and LSD-test when appropriate (for comparing multiple groups). $P<0.05$ was considered significant.

2 Results

2.1 Changes in fasting body mass, blood glucose and serum insulin levels, and insulin resistance in T2DM mice

To identify whether Art can improve T2DM, a T2DM mouse model was first established. In the forth week, fasting body mass were significantly increased in the mice fed on HFD as compared with the control ones, but began to reduce significantly upon STZ injection (Figure 1a). In the sixth week, T2DM model mice exhibited a remarkable increase in fasting blood glucose level and reduction of body mass as compared with the control mice, respectively (Figure 1a, b). Those results indicated that a mouse model of T2DM was established[19].

In the next 4 weeks, fasting body mass of T2DM group was still lowering as compared with the control group, while blood glucose level was still rising (Figure 1a, b). However, after 4 weeks drug treatment, fasting body mass significantly increased in T2DM+ Art group as compared with T2DM group, but fasting blood glucose level notably declined. Furthermore,

T2DM group mice had a higher serum insulin level and insulin resistance index value than that of the control group mice (Figure 1c, d). As expected, Art treatment obviously normalized serum insulin level and decreased insulin resistance index of T2DM mice. These results confirm that Art has an eminent role in improving insulin resistance index.

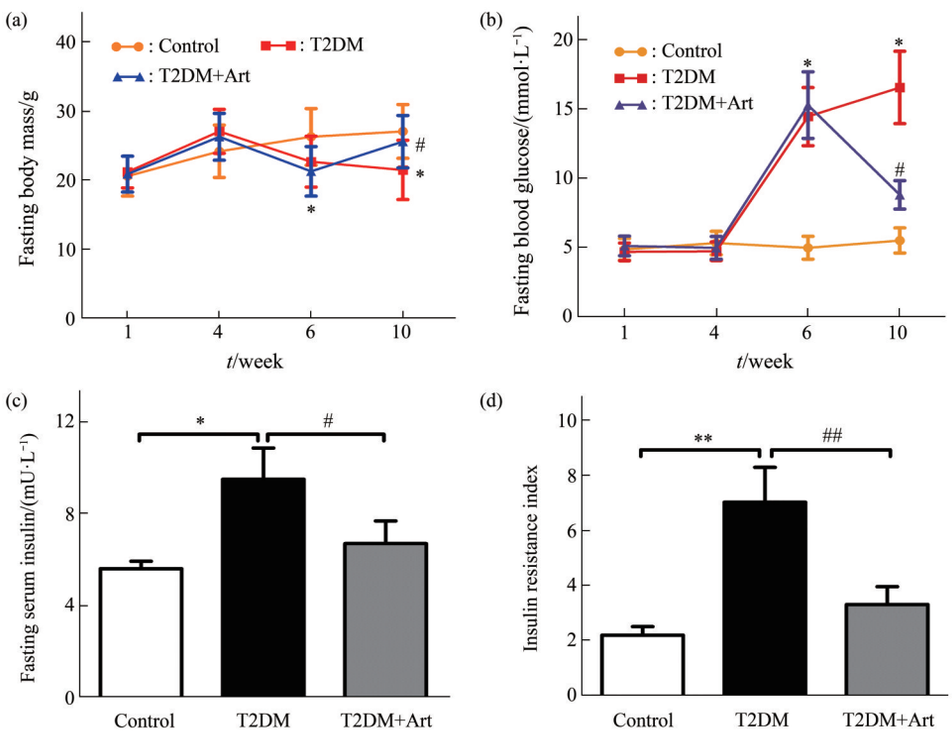

Fig. 1 The effect of artemisinin on fasting body mass and blood glucose level, serum insulin content, and insulin resistance index of T2DM mice

(a) Changes in body mass of the mice during the 10 weeks. (b) Fasting blood glucose levels of mice in each group (n=10−13). (c) Serum insulin levels were measured by commercial kits (n=10−13). (d) Insulin resistance index was calculated (n=10−13). Data are mean ± SEM. *P < 0.05, **P < 0.01 *vs* the control group; #P < 0.05, ##P < 0.01 *vs* T2DM group.

2.2 Art ameliorates cognitive function deficit in T2DM mice

To clarify whether Art has the ability to improve cognitive function of T2DM mice, we explored the influences of this medicine on the learning and memory performance of the mice by the NOR test, Y maze test, and MWM test.

In the NOR test, we found no significant difference (Figure 2a) in total exploration time between groups during testing session, indicating that there was no notable difference in activity ability and activity degree between the groups. As expected, recognition index was significantly declined in T2DM group as compared with that in normal control group (Figure 2b). However, this decline in recognition index of T2DM mice was profoundly ameliorated by Art treatment. These results indicate that Art indeed improves NOR cognitive performance in T2DM mice, but does not affect their spontaneous exploratory behaviors.

In Y maze test, we demonstrated that no significant difference (Figure 2c) in total entry times among groups. For alteration rate, there was a notable difference between groups. As shown in Figure 2d, the alteration rate of T2DM group was significantly decreased when compared with the normal control group. In contrast, this decrease was ameliorated by Art treatment. These findings reveal that Art improves the spatial working memory in T2DM in the Y maze test, but does not influence their locomotor ability.

Following the Y maze analysis, we further investigated the beneficial effect of Art on cognitive

function of T2DM mice using MWM test. As shown in Figure 2e, the escape latency of each group to reach the hidden platform reduced over 4 days training period. As compared with the control group, the swimming routes were significantly complex and the escape latency to find the hidden platform obviously prolonged in T2DM group when compared with the control group. Moreover, average time taken in the target quadrant and times across the platform in T2DM group remarkably decreased when compared with the normal control group (Figure 2f, g). As expected, Art treatment not only simplified the swimming routes and shortened the escape latency to the platform, but also significantly increased times across the platform. These results indicate that Art ameliorates MWM memory impairment in T2DM mice.

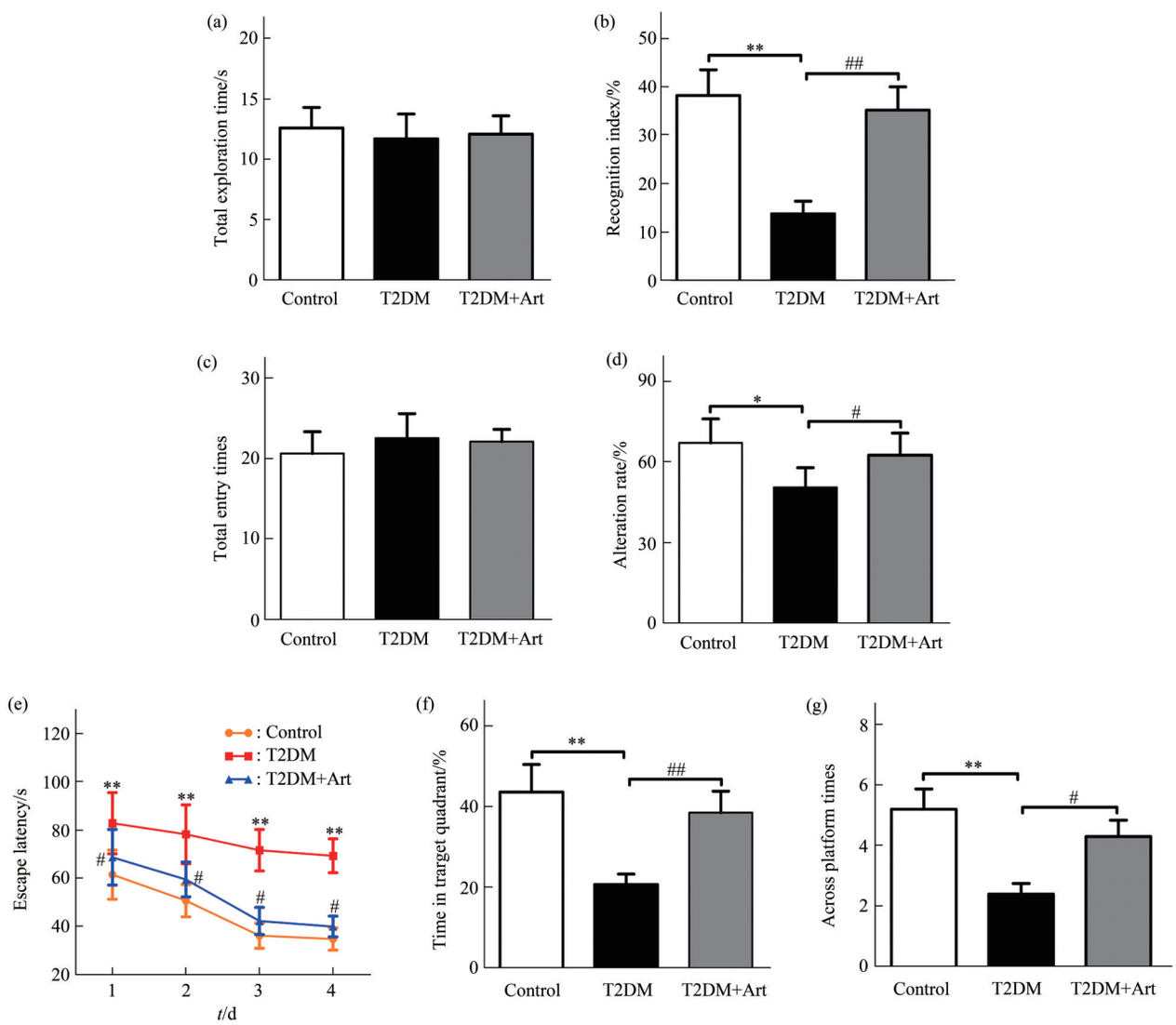

Fig. 2　Artemisinin improves the learning and memory ability of T2DM mice in the NOR, Y maze and WMW tests

In the NOR test, total time spent exploring objects (a) and recognition index (b) were analyzed. In the Y maze test, total times entering arms (c) and the alternation ratio (d) were analyzed. In the WMW test, the escape latency (e) to find the platform in the navigation phase, time spent in the target quadrant (f) and times crossing platform (g) during probe trail phase were also analyzed. Data are mean±SEM (n=10−13). *P < 0.05, **P < 0.01 *vs* the control group; #P < 0.05, ##P < 0.01 *vs* T2DM group.

2.3 Art increases the expression of PI3K and elevates the ratio of p−Akt to Akt in the hippocampus of T2DM mice

Considering activation of PI3K/Akt signaling can ameliorate hippocampal neuronal injury and cognition deficits in diabetes[24], Western blot was used to measure the expression of PI3K and p-Akt in the hippocampus of T2DM mice treated with Art. As shown in Figure 3a, c, the protein level of PI3K in the hippocampus of diabetic mice exhibited a significant decline when compared with that in the normal control ones. Consistent with the decline in PI3K expression, a significant decrease in the ratio of p-Akt/Akt (Figure 3b, d) was measured in the hippocampus of T2DM mice, indicating the PI3K/Akt signaling pathway was inhibited in the diabetic mice. In contrast, Art treatment group had a significant increase both in protein levels of PI3K and p-Akt as compared with that in non-treated diabetic group. These results indicate that Art can restore the decline in activation of PI3K/AKT signaling in the hippocampus of T2DM mice.

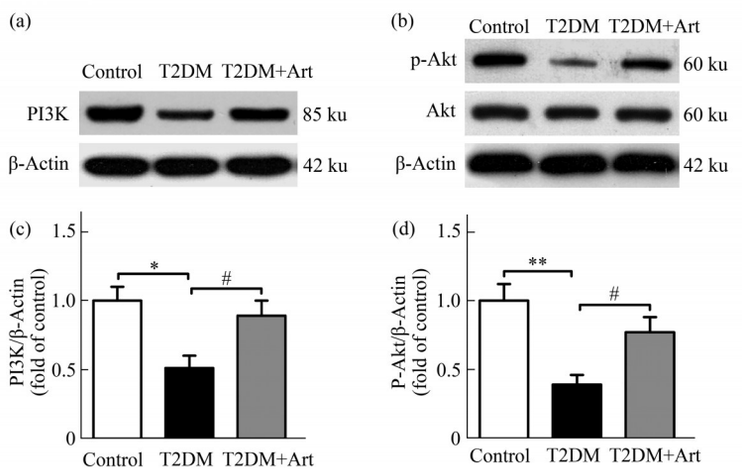

Fig. 3 Artemisinin up−regulates PI3K and p−Akt protein levels in the hippocampus of T2DM mice

Diabetic mice were administrated with artemisinin (Art, 40 mg/kg/d) or equal volume of PBS by intraperitoneal injection for 4 weeks. (a, b) Western bolt was used to measure the protein levels of PI3K, Akt and p-Akt (Ser 473). (c, d) Densitometry analysis of PI3K and p-Akt protein levels was performed by the Bio-Rad software. β-Actin was used as control for protein loading. The data are expressed as mean±SEM (n=3). *P < 0.05, **P < 0.01 *vs* the control group; #P < 0.05 *vs* T2DM group.

2.4 Art increases SYN and PSD−95 protein expression in the hippocampus of T2DM mice

The activation of PI3K/Akt pathway enhances synaptic activity *via* up-regulating SYN and PSD-95 protein levels[25]. Consequently, we further clarified the molecular mechanism of Art attenuating cognitive decline in diabetic mice, the expression levels of these two synaptic proteins in the hippocampus of T2DM mice were detected by Western blot. As shown in Figure 4a−c, the protein levels of SYN and PSD-95 in the hippocampus of T2DM mice were less than those in the normal control ones, indicating the impaired synaptic activity in the diabetic mice. However, the decreases in these two synaptic protein levels in T2DM mice were reversed by Art treatment. These results suggest that Art improves cognition in T2DM mice may be *via* promoting the expression of SYN and PSD-95 in hippocampus.

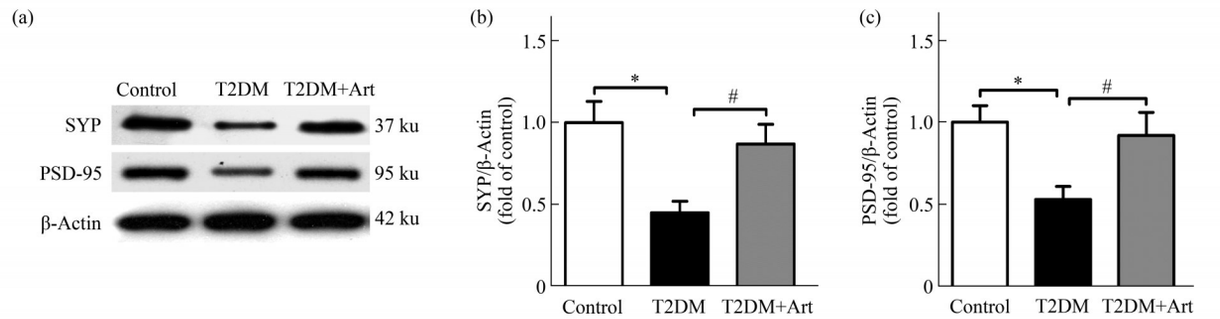

Fig. 4 Artemisinin increases the expression of PSD−95 protein in the hippocampus of T2DM mice

Diabetic mice were administrated with artemisinin (Art, 40 mg/kg/d) or equal volume of PBS by intraperitoneal injection for 4 weeks. (a) Western bolt was performed to detect the protein levels of SYN and PSD-95. (b, c) The relative optical density values of SYN and PSD-95 were quantified with Bio-Rad software. β-Actin was used as control for protein loading. The data are expressed as mean±SEM (n=3). *P < 0.05 *vs* the control group; #P < 0.05 *vs* T2DM group.

2.5 Art ameliorates neuronal injury in the hippocampal CA1 region of the mice with T2DM mice

Hippocampal neuronal damage is a major pathological feature that is involved in cognitive impairment in diabetes[26]. To reveal whether Art can attenuate hippocampal neuronal injury in CA1 area of the mice with T2DM, the pathomorphology and number alterations of pyramidal neurons in this region were evaluated by H&E staining. As shown in Figure 5a, surviving pyramidal neurons in CA1 area appeared as big and round nuclei, while the impaired neurons presented with shrunken and karyopyknosis. Compared with mice in the normal control group, pyramidal neurons in T2DM group sparsely arranged and exhibited shrunken and pyknotic nucleus, and cell number significantly reduced (Figure 5b), indicating hippocampal neuronal damage and loss were occurred in T2DM mice. Interestingly, these abnormal changes in T2DM mice were significantly reversed by Art treatment. These results demonstrate that Art improves neuronal morphology and promotes neuronal survival in the hippocampal CA1 area of T2DM mice.

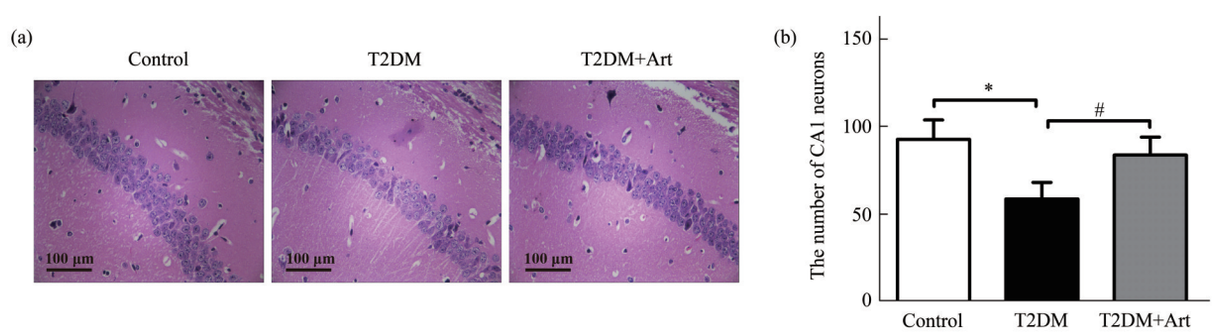

Fig. 5 Artemisinin attenuates cell loss and improves neuronal morphology in the hippocampal CA1 region of T2DM mice

Diabetic mice were administrated with artemisinin (Art, 40 mg/kg/d) or equal volume of PBS by intraperitoneal injection for 4 weeks. Changes in neuron loss and neuronal morphology in the hippocampal CA1 area were observed by H&E staining. (a) Representative light micrographs of H&E-stained neurons in the CA1 hippocampus of the mice. (b) Statistical analyses for neuron number in the CA1 hippocampus of the mice. Each group n= 9/3 (brain sections/mice). The data are expressed as mean±SEM. *P < 0.05 *vs* the control group; #P < 0.05 *vs* T2DM group.

2.6 Art reverses synaptic loss and ultrastructure impairment in CA1 area of T2DM mice

Hippocampal synaptic loss and synaptic plasticity impairment are responsible for cognitive defects in diabetes[27]. To further confirm the effect of Art on the synaptic plasticity in T2DM mice, synaptic

ultrastructure parameters such as synaptic density, synaptic cleft width, synaptic curvature, and PSD thickness in CA1 region of the mice were examined by TEM. As shown in Figure 6, compared with the normal control mice, T2DM mice exhibited significant decreases in synaptic density, synaptic curvature and PSD thickness, and an obvious increase in synaptic gap width in CA1 area. As expected, Art treatment increased the number of synapses, and synaptic curvature, as well as augmented the thickness of PSD, while notably narrowed the width of synaptic cleft. These results indicate that Art indeed rescues hippocampal synaptic loss and synaptic plasticity impairment in T2DM mice.

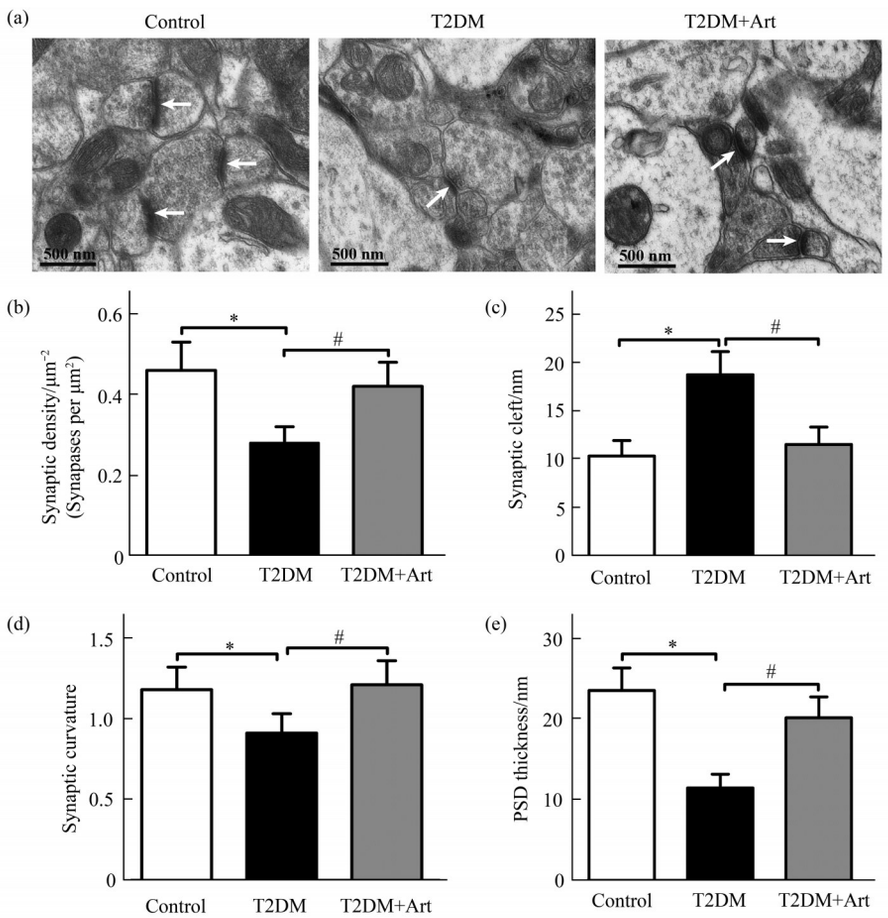

Fig. 6 Artemisinin improves synaptic ultrastructures in the CA1 area of T2DM mice

Diabetic mice were administrated with artemisinin (Art, 40 mg/kg/d) or equal volume of PBS by intraperitoneal injection for 4 weeks. Changes in synaptic ultrastructures in the CA1 hippocampus of the mice were observed by TEM. (a) Representative images of the synaptic ultrastructures in the hippocampal CA1 region. The white arrow indicates synapse. (b) The density of synapse in CA1. Ten electron micrographs representing 283.68 μm^2 neuropil areas in each mouse were used for quantification. Total synapse density was expressed as the number of synapses per μm^2 of neuropil. n=3. (c−e) The statistical data of the synaptic gap width, synaptic curvature, and PSD thickness in CA1 region. Ten synapses were calculated from each mouse and each group contained three mice. The data are expressed as mean±SEM. *P < 0.05 *vs* the control group; #P < 0.05 *vs* T2DM group.

3 Discussion

The present study aimed to clarify the role and underlying mechanisms of Art on cognitive performances in T2DM mice. Our results manifested that Art administration profoundly ameliorated cognitive deficits in the diabetic mice. Mechanically, Art treatment attenuated neuronal damage and restored synaptic plasticity in CA1 area of diabetic mice, which was possibly due to the activation of PI3K/Akt. Collectively, these findings suggested Art prevents cognitive decline in T2DM mice may be *via* activating PI3K/Akt pathway to rescue hippocampal synaptic plasticity (Figure 7).

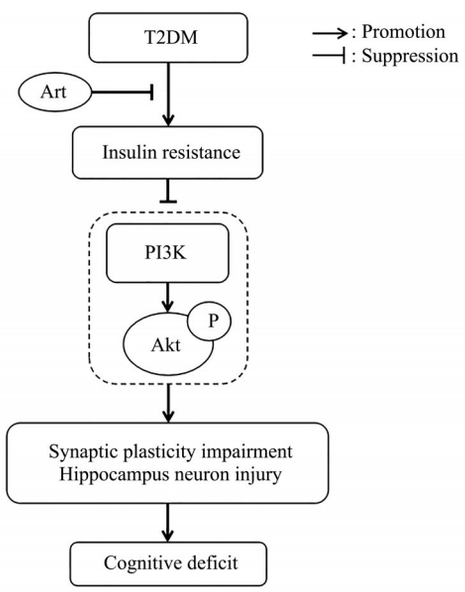

Fig. 7 Proposed mechanism by which Art improves T2DM−associated cognitive decline

T2DM decreases the activity of PI3K/Akt insulin signaling pathway in the hippocampus. The inactivation of this signaling further leads to hippocampal neuron damage and synaptic plasticity impairment, which ultimately results in cognitive deficit. Treatment with artemisinin (Art, 40 mg/kg/d) improves T2DM-associated cognitive decline through restoring the activation of the insulin signaling pathway to protect hippocampal neuron from injury and synaptic plasticity impairment.

Increasing evidences indicate that patients with T2DM are prone to cognitive decline, even developing into dementia[1, 28]. Although the incidence of cognitive deficits is much higher in diabetic individuals, there is no available method to prevent this severe complication. Therefore, finding new therapeutic strategies for T2DM-induced cognitive impairment are crucial. Recently, Art and its derivatives have been demonstrated to be effective agents for ameliorating diabetes-associated neuronal damage and rescuing isoflurane-induced cognitive impairments in rats, but their effect on learning and memory in diabetes is unclear[29]. Hence, in this study, we first investigated the role of Art on cognitive performances of T2DM mice induced by STZ and HFD. Our results indicated that cognitive functions in T2DM mice significantly declined. However, this decline was reserved by Art administration, as evidenced by an increase in recognition index in the NOR test and an increase in alternation performance in the Y maze test, and a reduction in escape latency and an increase in the crossing platform times in the MWM test. These results assure that Art can ameliorate cognitive impairment in T2DM mice.

Learning and memory are closely associated with the hippocampal CA1 area, which is responsible for information processing[30]. Given neuronal loss and structure injury in this region causes impairment in cognitive performances[31], strategies to ameliorate neuronal damage in CA1 region are a potential therapy for preventing cognitive deficits. Furthermore, considerable data confirm that hippocampal CA1 neurons are more vulnerable to hyperglycemia in diabetic conditions. To identify whether Art improved cognitive functions of T2DM mice *via* attenuating neuronal injury, neuronal morphologic changes in this hippocampal area were evaluated by H&E staining. Our results shown that the number of CA1 hippocampal neurons was reduced and the structure was impaired in T2DM mice, which are in agreement with previous studies[3, 22]. Excitingly, correlated with changes in blood glucose and insulin levels, the neuronal damage in hippocampal CA1 region of T2DM mice treatment with Art was remarkably alleviated, as evidenced by an increase in the number of neurons and the improvement of neuronal morphology. Collectively, these results reveal that Art has beneficial effect on diabetes-associated cognitive decline through protecting hippocampal neurons against hyperglycemia.

PI3K/Akt pathway, a major insulin signaling pathway, which plays a critical role in promoting neuronal survival and improving cognitive function[32]. To further clarify the underlying mechanism by which Art protects T2DM mice against cognitive impairment, the effect of this medicine on PI3K/Akt activation in the hippocampus was evaluated. Our results shown that the total PI3K protein expression and p-Akt levels were dramatically reduced, indicating that PI3K/Akt pathway was inhibited in the hippocampus of T2DM mice. Notably, Art treatment restored the activity of this pathway, as indicating by the elevated the levels of total PI3K protein and p-Akt in the hippocampus of T2DM mice. In addition, Lin *et al.* [33] demonstrated that Art protected HT-22 hippocampal neurons against glutamate-induced cell death through activating this pathway. Hence, these data suggest that Art ameliorates diabetic cognitive defect of mice maybe

via activating PI3K/AKT pathway.

PI3K/AKT activation enhances the expression of SYN and PSD-95 protein, which makes a great contribution to improving synaptic plasticity and cognitive performance[25]. Studies have reported that lower levels of SYN and PSD-95 protein levels leads to abnormal alterations in synaptic ultrastructures such as the width of the synaptic cleft, synaptic curvature, and PSD thickness[23-34]. Furthermore, a decrease in SYN and PSD-95 expression has been shown in various neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and diabetic cognitive defects[35-37]. Therefore, we further investigated whether Art protects T2DM mice against impairment in synaptic plasticity. Our results uncovered that synaptic plasticity was severely impaired in the hippocampus of T2MD mice, as indicted by the increased synaptic cleft width, and the decreased SYN and PSD-95 expression, PSD thickness and synaptic curvature. These abnormal alterations of synaptic plasticity in the hippocampus could directly cause the obstacle of synaptic signal transduction, which may be the reason why the diabetic mice manifested poor cognitive function. Interestingly, Art obviously improved synaptic plasticity in the hippocampus of T2DM mice. In accordance with the improvement of hippocampal synaptic plasticity, cognitive performances of T2DM mice treated with Art were improved. These results imply that Art prevents diabetic mice from synaptic plasticity deficit, thereby ameliorating cognitive decline.

4 Conclusion

The present study revealed that Art significantly ameliorated cognitive deficit in T2DM mice. The underlying mechanism may involve in the improvement of hippocampal synaptic plasticity *via* activating PI3K/Akt pathway in the diabetic mice. These findings suggest that Art may be a promising therapeutic agent for diabetic cognitive deficits.

Referee Prof. TANG Chao-Ke (Editorial Board Member of PIBB)

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青蒿素经PI3K/Akt通路改善突触可塑性减轻糖尿病小鼠认知障碍*

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摘要　目的　本研究旨在阐明青蒿素对II型糖尿病（T2DM）小鼠认知功能障碍的改善作用及其机制。**方法**　C57BL/6J小鼠单次腹腔注射STZ（100 mg/kg）后联合高脂饲料喂养建立T2DM模型。T2DM小鼠随后腹腔注射青蒿素（40 mg/kg/d）或等体积溶剂。干预4周后，新物体识别、Y迷宫和Morris水迷宫实验检测小鼠的学习和记忆能力。蛋白质印迹法（Western blot）检测海马PI3K、Akt、磷酸化Akt、SYN和PSD-95蛋白的表达。透射电镜观察海马CA1区突触密度和突触超微结构改变。**结果**　与模型组相比，青蒿素干预组T2DM小鼠的认知功能显著改善，海马中PI3K和磷酸化Akt水平升高，SYN和PSD-95蛋白表达增加，CA1区神经元丢失减少。此外，青蒿素干预组小鼠CA1区的突触密度、PSD-95和突触界面曲率增加，突触间隙宽度减小。**结论**　青蒿素可能通过激活海马PI3K/Akt途径增强突触可塑性，从而减轻T2DM小鼠认知功能障碍；青蒿素有望成为治疗糖尿病性认知功能障碍的新型药物。

关键词　糖尿病性认知功能障碍，突触可塑性，海马，青蒿素，PI3K
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