Evodiamine improves cognitive abilities in SAMP8 and APP<sup>swe</sup>/PS1<sup>ΔE9</sup> transgenic mouse models of Alzheimer’s disease

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Aim: To investigate the effect of evodiamine (a quinolone alkaloid from the fruit of *Evodia rutaecarpa*) on the progression of Alzheimer’s disease in SAMP8 and APP<sup>swe</sup>/PS1<sup>ΔE9</sup> transgenic mouse models.

Methods: The mice at age of 5 months were randomized into the model group, two evodiamine (50 mg·kg<sup>-1</sup>·d<sup>-1</sup> and 100 mg·kg<sup>-1</sup>·d<sup>-1</sup>) groups and an Aricept (2 mg·kg<sup>-1</sup>·d<sup>-1</sup>) group. The littermates of no-transgenic mice and senescence accelerated mouse/resistance 1 mice (SAMR1) were used as controls. After 4 weeks of treatment, learning abilities and memory were assessed using Morris water-maze test, and glucose uptake by the brain was detected using positron emission tomography/computed tomography (PET/CT). Expression levels of IL-1β, IL-6, and TNF-α in brain tissues were detected using ELISA. Expression of COX-2 protein was determined using Western blot.

Results: In Morris water-maze test, evodiamine (100 mg·kg<sup>-1</sup>·d<sup>-1</sup>) significantly alleviated the impairments of learning ability and memory. Evodiamine (100 mg·kg<sup>-1</sup>·d<sup>-1</sup>) also reversed the inhibition of glucose uptake due to development of Alzheimer’s disease traits in mice. Furthermore, the dose of evodiamine significantly decreased the expression of IL-1β, IL-6, TNF-α, and COX-2 that were involved in the inflammation due to Alzheimer’s disease.

Conclusion: The results indicate that evodiamine (100 mg·kg<sup>-1</sup>·d<sup>-1</sup>) improves cognitive abilities in the transgenic models of Alzheimer’s disease.

Keywords: evodiamine; Alzheimer’s disease; imaging; Morris water-maze test; SAMP8; APP<sup>swe</sup>/PS1<sup>ΔE9</sup>; inflammation
mutant amyloid precursor protein and presenilin 1 (APP<sup>swt/PS1<sub>ΔE9</sub></sup>) and senescence-accelerated mouse/prone 8 (SAMP8) transgenic mice mimic the AD phenotype via different mechanisms. The APP<sup>swt</sup> transgene encodes a mouse-human hybrid with the mouse sequence in the extracellular and intracellular regions and a human sequence within the Aβ domain with Swedish mutations K594N/M595L. The PS1<sup>ΔE9</sup> transgene encodes the exon 9-deleted human presenilin-1. The APP<sup>swt/PS1<sub>ΔE9</sub></sup> double transgenic mice develop behavioral, phenotypic, and pathological features which make them useful as an AD model<sup>[9]</sup>. The senescence-accelerated mouse (SAM) is an accelerated aging model that was established through phenotypic selection from a common genetic pool of the AKR/J strain of mice. The SAMP8 mice show significant impairments in a variety of learning tasks, when compared with senescence accelerated mouse/resistance 1 (SAMR1) mice. Moreover, the abnormal APP and Aβ metabolism in the SAMP8 mice brain suggests that SAMP8 is not only a good model for studying age-related learning and memory deficits, but may also prove to be a useful model for studying Aβ-mediated effects in cognitive decline<sup>[10–12]</sup>.

Chinese herbs have been and still are widely used as important remedies in Oriental medicine. Over recent years, a variety of biologically active constituents have been isolated from these sources. It is reported that tea polyphenol can reverse scopolamine- or D-galactose-induced deficits in cognitive abilities<sup>[13, 14]</sup>. Resveratrol acts to reduce pathological plaques in mice<sup>[15, 16]</sup> and has anti-oxidant activity <em>in vitro</em> and <em>in vivo</em><sup>[16, 17]</sup>. Evodiamine, a quinolone alkaloid, is a component isolated from the fruit of <em>Evodia rutaecarpa</em>, a traditional Chinese herb that has been used for treatment of headaches, abdominal pain, postpartum hemorrhage, dysentery and amenorrhea<sup>[18, 19]</sup>. However, the treatment of AD with evodiamine has not been reported. In the present study, we investigated the effect and the possible mechanisms of action of evodiamine in mouse models of AD.

**Materials and methods**

**Animal models**

The APP<sup>swt/PS1<sub>ΔE9</sub></sup> double-transgenic mouse in a C57BL/6J genetic background was bred in our laboratory. This mouse shows spatial memory deficits at 3 months of age and senile plaques in brain tissue at 4.5 months of age<sup>[9]</sup>. SAMP8 and SAMR1 mouse were obtained from the Department of Laboratory Animal Science, Peking University Health Science Center (Beijing, China). SAMR1 is one species of SAM, which shows normal aging characteristics. SAMP8 is a model of age-related dementia of the Alzheimer type and shows significant impairment in a variety of learning tasks<sup>[10–12]</sup>. All mice were maintained in an AAALAC-accredited facility and the use of animals was approved by the Animal Care and Use Committee of the Institute of Laboratory Animal Science of Peking Union Medical College (SCXK-2005-0013).

**Groups and treatment**

For screening of 12 herbal monomers, 5 month old transgenic mice were randomly divided into 13 groups (n=16 to 20 per group). One group was used as a vehicle control group and the remaining 12 groups were used for treatment with herbal monomers. The herbal monomers were purchased from Qingdao University Natural Product Institute (Qingdao, China). The littermates of non-transgenic mice were used as wild type (WT) controls. The WT and vehicle groups received a standard diet and the treated groups received a standard diet plus the respective monomers at the doses described in Table 1. The dosages of 12 individual herbal monomers were selected based on human-equivalent dosages and data in previous reports<sup>[13, 14, 16, 20]</sup>. Morris water-maze tests were performed after 4 weeks of treatment, since this time period was believed sufficient for APP<sup>swt/PS1<sub>ΔE9</sub></sup> transgenic mouse to show significant impairments in function when compared with WT mice.

For evodiamine analysis, APP<sup>swt/PS1<sub>ΔE9</sub></sup> and SAMP8 transgenic mice at 5 months of age were randomized into vehicle, evodiamine-Evo 50 (50 mg kg<sup>−1</sup> d<sup>−1</sup>), evodiamine-Evo 100 (100 mg kg<sup>−1</sup> d<sup>−1</sup>) and Aricept (2 mg kg<sup>−1</sup> d<sup>−1</sup>) groups. Aricept is an inhibitor of acetyl-cholinesterase and is presently used in long-term symptomatic treatments for patients with AD, since it enhances CNS levels of synaptic acetyl-choline. The administered does of Aricept was the human-equivalent dosage, calculated according to the weights of the mice. The littermates of no-negative mice and SAMR1 mice were used as WT controls. The mice were treated with standard diet or standard diet plus monomer for 4 weeks and their capacities for learning and memory were assessed by the Morris water-maze test.

**Morris water-maze test**

The protocol of the Morris water-maze test was modified from the reported methods<sup>[21–23]</sup>. Briefly, the apparatus included a pool with a diameter of 100 cm, filled with opaque water at 22–24 °C. An escape platform (15 cm in diameter) was placed 0.5 cm below the water surface. Two tests, constituting two blocks of trials, 60 s each, were performed daily for 5 consecutive days. The platform location and the animal starting point were held constant within each pair of daily tests, but they were changed from day to day. The mice were allowed to stay on the platform for 15 s before and after each trial. The time taken for an animal to reach the platform (latency period) was recorded. On the fifth day, a probe test was performed after the second daily trial; briefly, the platform was removed from the maze, and the number of crossings by the mice when the area from which the platform had been removed was recorded (for a maximal period of 60 s). Monitoring was performed with a video tracking system (Noldus Ltd, Ethovision XT, Holland). Results are represented as mean±SEM.

**Histochemical analysis**

The brain tissues from 6 month old animals were fixed in neutral buffered formalin. The tissues were dehydrated in an alcohol gradient then embedded in paraffin and sliced at 4 μm thickness. Thioflavain-S staining was performed on these
1.5% isoflurane, along with 31% O\textsubscript{2} groups were randomly chosen. They were anesthetized using 1.5% isoflurane, along with 31% O\textsubscript{2} inhalation (flow rate: 2.5 L/min) through a nose cone prior to injection of the tracer. During operation, the body temperature of each mouse was maintained by a thermostat-controlled thermal heater; the mouse was imaged on a small-animal scanner (microPET/CT, Inveon, Siemens). Prior to the dynamic small-animal procedure, a 10-min PET scan, and a 10-min CT scan was obtained for attenuation correction of small-animal PET images. Images were reconstructed using the filtered back-projection algorithm with CT-based photon-attenuation correction\[26\]. The voxel size was 0.2×0.2×0.8 mm\textsuperscript{3}.

**PET/CT images analysis**

The PET/CT scan was modified from the reported method\[25\]. Briefly, mice from treated and positive and negative control groups were randomly chosen. They were anesthetized using 1.5% isoflurane, along with 31% O\textsubscript{2} inhalation (flow rate: 2.5 L/min) through a nose cone prior to injection of the tracer. During operation, the body temperature of each mouse was maintained by a thermostat-controlled thermal heater; the mouse was imaged on a small-animal scanner (microPET/CT, Inveon, Siemens). Prior to the dynamic small-animal procedure, \textsuperscript{18}F-FDG tracer (FDG) (at ~14.8–18.5 MBq) was injected as a bolus (~200 μL) through a tail vein catheter and the animal was kept at room temperature for 45 min. FDG is a glucose analog that is actively transported into cells. Then the mouse was exposed to a 10-min PET scan, and a 10-min CT scan was obtained for attenuation correction of small-animal PET images. Images were reconstructed using the filtered back-projection algorithm with CT-based photon-attenuation correction\[26\]. The voxel size was 0.2×0.2×0.8 mm\textsuperscript{3}. The field of view was 11.28×12.66 cm\textsuperscript{2}.

**ELISA**

Mouse brain tissue was sampled and 100 mg of tissue per animal was homogenized in 1.0 mL of 0.9% NaCl solution containing 0.1% PMSF (Sigma, MO, USA). After centrifugation at 14000 rpm for 15 min at 4 °C, the resulting supernatants were washed in 50% ethanol and in water, then dried, and dipped in Histo-Clear before being cover-slipped with Permount. All chemicals were obtained from Sigma.

**Western blot**

Mouse brain tissue was sampled and 100 mg of tissue per animal was homogenized in 1.0 mL of RIPA buffer containing 0.1% PMSF and 0.1% protease inhibitor cocktail (Sigma, MO, USA). After centrifugation at 14000 round per minute for 15 min at 4 °C, the protein concentration in the resulting supernatants was detected by the BCA method. Aliquots of 60 μg per sample were subjected to 10% SDS-PAGE, followed by transfer onto a nitrocellulose membrane (Immobilon NC; Millipore, Molsheim, France). Immunoblotting was then carried out with antibodies specific for COX-2 at 1:100 dilution (Cayman Chemical, USA). Primary antibodies were visualized with anti-rabbit HRP-conjugated secondary antibodies (Santa Cruz) using a chemiluminescence detection system (Western Blotting Luminal Reagent, Santa Cruz). Sample loading was normalized with GAPDH. Bands were quantified by the densitometry function of the Quantity One software.

**Statistical analyses**

Statistical analyses were performed by one-way ANOVA followed by Tukey’s Honestly Significantly Different (HSD) test. Data with a \( P<0.05 \) were deemed statistically significant. Results are expressed as mean±SEM.

**Results**

**Comparative analysis of the effects of 12 different herbal monomers in APP\textsuperscript{sw} /PS1\textsuperscript{ΔE9} transgenic mice**

Twelve herbal monomers, that have varied effects on the CNS, microcirculation, anti-oxidative or anti-inflammatory responses, were selected to analyze their respective effects on cognitive abilities of APP\textsuperscript{sw} /PS1\textsuperscript{ΔE9} transgenic mice. These herbal monomers and their usages are illustrated by Table 1, which shows their individual effects as evaluated by the Morris water-maze test. Only evodiamine showed the capacity to significantly improve cognitive abilities of APP\textsuperscript{sw} /PS1\textsuperscript{ΔE9} transgenic mice, specifically referring to their spatial memory deficits at 3 months of age and the AD phenotype which progressively develops as the animals age.

**Table 1. Screening of herbal monomers with the Morris water-maze test.** Twelve herbal monomers were selected for preliminary treatment of APP\textsuperscript{sw} /PS1\textsuperscript{ΔE9} transgenic mice with the indicated doses and their effects were evaluated by the Morris water-maze test. Latency on the third day of the tests and platform crossings on the day after the five-day training period were recorded. (\( P>0.05, \) \( P<0.05, \) \( P<0.01 \) vs vehicle group).

| Group          | Dose (mg·kg\textsuperscript{-1}·d\textsuperscript{-1}) | Latency (s) | Platform crossings (count) | Number of animals |
|----------------|--------------------------------------------------------|-------------|----------------------------|------------------|
| WT             | –                                                      | 10.62±2.01\textsuperscript{a} | 5.20±0.81\textsuperscript{a} | n=20              |
| Vehicle        | –                                                      | 21.23±3.48\textsuperscript{b} | 3.75±0.53\textsuperscript{b} | n=16              |
| Curinine       | 80                                                     | 18.58±1.7\textsuperscript{b}  | 4.75±1.11\textsuperscript{b} | n=16              |
| Evodiamine     | 100                                                    | 12.18±0.3\textsuperscript{b}  | 6.63±1.15\textsuperscript{b} | n=16              |
| Tea polyphenols| 100                                                    | 15.05±3.50\textsuperscript{b} | 4.89±0.54\textsuperscript{b} | n=16              |
| Chuanxiongine  | 50                                                     | 14.17±3.50\textsuperscript{a} | 4.75±0.92\textsuperscript{a} | n=18              |
| Polydatin      | 100                                                    | 20.94±3.34\textsuperscript{a} | 3.89±0.75\textsuperscript{a} | n=18              |
| Tanshinone IIA | 100                                                    | 14.65±2.5\textsuperscript{a}  | 3.67±0.53\textsuperscript{a} | n=18              |
| Astragaloside  | 100                                                    | 14.67±2.1\textsuperscript{a}  | 2.89±0.54\textsuperscript{a} | n=16              |
| Puerarin       | 100                                                    | 18.59±3.3\textsuperscript{a}  | 2.88±0.35\textsuperscript{a} | n=20              |
| Salvianic acid A| 100                                                    | 14.62±2.4\textsuperscript{a}  | 3.78±0.57\textsuperscript{a} | n=20              |
| Quercetin      | 100                                                    | 24.17±3.03\textsuperscript{a} | 5.00±0.54\textsuperscript{a} | n=20              |
| Resveratrol    | 150                                                    | 15.64±2.94\textsuperscript{a} | 3.60±0.76\textsuperscript{a} | n=20              |
| Tetrahydropalmatine| 100                                                     | 20.22±3.80\textsuperscript{a} | 3.80±0.44\textsuperscript{a} | n=16              |

**Evodiamine treatment increased spatial learning and memory in SAMP8 and APP\textsuperscript{sw} /PS1\textsuperscript{ΔE9} transgenic mice**

To investigate the effect of evodiamine on AD models, it was used to treat both APP\textsuperscript{sw} /PS1\textsuperscript{ΔE9} and SAMP8 transgenic mice at age of 5 months. In consideration of the welfare of the animals, administration of evodiamine was effected through oral administration via their diet. After 4 weeks of treatment, learning capacity and memory were assessed by the Morris water-maze test (Figure 1A-1D). During training d 1 the
Evodiamine treatment also resulted in improvement in the behavior of the SAMP8 mice, evinced by a decrease in the latency period of 43% ($P<0.05$, $n=12$) (Figure 1C) and by a significantly increased number of crossings of the original platform area equivalent to 42% ($P<0.05$, $n=12$) in the Evo 100 group (Figure 1D), compared with observations for the vehicle group.

Treatment with evodiamine at a dose of 50 mg·kg$^{-1}$·d$^{-1}$ showed no improvement in the behavior of the SAMP8 mice. Thus, the administration of the dose of 100 mg·kg$^{-1}$·d$^{-1}$ was significantly effective in improvement of behavior in both the SAMP8 and APPswe/PS1$^{ΔE9}$ transgenic mouse models, which develop AD characteristics via different pathological mechanisms.$^{9,12}$

### Evodiamine had no effect on Aβ deposition in the APPswe/PS1$^{ΔE9}$ transgenic mouse

Since the dose of 100 mg·kg$^{-1}$·d$^{-1}$ was effective in both SAMP8 mice and APPswe/PS1$^{ΔE9}$ mice, we used this dose to investigate whether evodiamine treatment would inhibit Aβ deposition in the APPswe/PS1$^{ΔE9}$ transgenic mouse, which forms senile plaque in brain tissue after 6 months of age.$^{9}$ After 4 weeks of administration, hippocampus tissues were sampled from the WT, vehicle, Evo 100, and Aricept groups. Paraffin sections were prepared and stained with thioflavin-S medium. Observation using the fluorescence microscope showed that Aβ deposition clearly occurred in the APPswe/PS1$^{ΔE9}$ transgenic mice compared with those in the WT control group. Neither evodiamine nor Aricept treatment showed observable inhibition of Aβ deposition in APPswe/PS1$^{ΔE9}$ transgenic mouse (Figure 2), suggesting that the effect of evodiamine on improvement of behavior in AD mouse models involves other mechanisms.

### Evodiamine treatment increased glucose uptake in brain tissue in the APPswe/PS1$^{ΔE9}$ transgenic mouse

The AD patient exhibits large decreases in glucose uptake and energy metabolism in the frontal cortex and temporal lobes.$^{1}$ Brain glucose uptake was detected by PET scan in living mice of the WT, vehicle, Evo 100, and Aricept groups after 4 weeks of treatment (Figure 3A, 3B). The results showed that glucose uptake by APPswe/PS1$^{ΔE9}$ transgenic mice was significantly decreased by 16% ($P<0.05$, $n=3$), compared with that of WT controls. Treatment with evodiamine ameliorated the glucose uptake decrease caused by APPswe/PS1$^{ΔE9}$ expression by 16% ($P<0.05$, $n=4$). Aricept administration also improved glucose uptake in APPswe/PS1$^{ΔE9}$ transgenic mice by 23% ($P<0.05$, $n=3$).

### Evodiamine treatment inhibited the expression of inflammatory cytokines in the APPswe/PS1$^{ΔE9}$ transgenic mouse

Inflammatory factors IL-1β, IL-6, and TNF-α were detected...
with the ELISA in lysates of brain tissues from WT, vehicle, Evo 100 and Aricept groups after 4 weeks of treatment (Figure 4). The results indicated that evodiamine decreased the levels of IL-1β by 23% ($P<0.05$), IL-6 by 27% ($P<0.05$), and TNF-α by 26% ($P<0.05$), compared with their levels in the vehicle group. Aricept administration also significantly decreased the levels of IL-1β and IL-6, but not that of TNF-α.

Figure 2. Evodiamine treatment has no effect on Aβ deposition in the brain of the APP$^{sw/PS1^{ΔE9}}$ transgenic mouse. After 4 weeks of administration, brain tissues from WT and vehicle mice, and mice treated with evodiamine at a dose of 100 mg·kg$^{-1}$·d$^{-1}$ (Evo 100) or with Aricept at a dose of 2 mg·kg$^{-1}$·d$^{-1}$ (Aricept) were utilized in standard pathological procedures and sections were stained with Thioflavin-S to visualize the deposition of Aβ. (Magnification×100).

Figure 3. Evodiamine treatment increases glucose uptake in the brain of the APP$^{sw/PS1^{ΔE9}}$ transgenic mouse. After 4 weeks administration, the WT and Vehicle mice, and those treated with evodiamine at a dose of 100 mg·kg$^{-1}$·d$^{-1}$ (Evo 100) or Aricept at a dose of 2 mg·kg$^{-1}$·d$^{-1}$ (Aricept) were subjected to the PET/CT scan to obtain typical images as shown in panel A. Glucose (as FDG) uptake per gram of brain tissue is depicted in panel B ($n=3–4$). ($^bP<0.05$ vs vehicle group).

Figure 4. Evodiamine treatment inhibits the expression of inflammatory cytokines in the APP$^{sw/PS1^{ΔE9}}$ transgenic mouse. After 4 weeks of administration, brain tissue from WT and Vehicle mice and those treated with evodiamine at a dose of 100 mg·kg$^{-1}$·d$^{-1}$ (Evo 100) or Aricept at a dose of 2 mg·kg$^{-1}$·d$^{-1}$ (Aricept) were sampled and total lysates were isolated. The levels of IL-1β (A, $n=6$), IL-6 (B, $n=6$), and TNF-α (C, $n=6$) were detected by ELISA kits. ($^bP<0.05$ vs vehicle group).

**Evodiamine treatment decreased the expression of COX-2 in the APP$^{sw/PS1^{ΔE9}}$ transgenic mouse**

COX-2 is one of the important determinants in inflammatory response-mediated cytotoxicity. Accumulation of COX-2 protein was observed in the vehicle group, compared with WT mice, and the accumulation of COX-2 protein caused by expression of APP$^{sw/PS1^{ΔE9}}$ transgenic genes was significantly reduced by evodiamine, by up to 73% ($P<0.01$, $n=4$) and reduced by Aricept by up to 67% ($P<0.01$, $n=4$) after 4 weeks of treatment (Figure 5A, 5B).

**Discussion**

In the present study we evaluated 12 herbal monomers in APP$^{sw/PS1^{ΔE9}}$ transgenic mice by the Morris water-maze test (Table 1). Evodiamine alone, from among the 12 herbal...
monomers, showed some effect on reversal of the AD phenotype through capacity to improve the cognitive abilities of the APP\textsuperscript{swe}/PS1\textsuperscript{ΔE9} transgenic mice.

We first showed that 4 weeks of administration of evodiamine to the APP\textsuperscript{swe}/PS1\textsuperscript{ΔE9} and SAMP8 transgenic mice improved spatial learning and memory of mice with symptoms of AD at 5 months of age (Figure 1). Our results indicate that evodiamine can improve spatial learning and memory in APP\textsuperscript{swe}/PS1\textsuperscript{ΔE9} and SAMP8 transgenic mice, but that the improvement is not due to reduction in the pathological development of senile plaque in brain tissues (Figure 2). The perturbations in energy metabolism in the AD patient evinced by large decreases in glucose uptake and energy metabolism in the frontal cortex and temporal lobes\cite{27} are also present in perturbations in energy metabolism in the AD patient evinced by large decreases in glucose uptake and energy metabolism in the frontal cortex and temporal lobes, and this basis that treatment with evodiamine could contribute to improvement of brain function in the AD model.

Evodiamine has been shown to have various effects on biological processes, such as testosterone\cite{28} and catecholamine secretion\cite{29}, as well as vasodilative\cite{30}, anti-nociceptive\cite{31}, obesity\cite{32}, and thermoregulatory and uterotonic effects\cite{33}. Evodiamine has anti-tumor potential through its ability to inhibit proliferation, induce apoptosis and reduce invasion and metastasis of a wide variety of tumor cells, including breast cancer, prostate cancer, leukemia, cancer, cervical cancer, colon cancer and lung cancer cells\cite{34}. Previous studies indicated that evodiamine represses COX-2 and inducible nitric oxide synthase (iNOS) expression and PGE2 release in RAW264.7 cells\cite{35} and inhibits LPS-induced NO production and iNOS up-regulation in microglial cells\cite{36}, suggesting that evodiamine has anti-inflammatory activity. Our results demonstrated that evodiamine decreased the levels of IL-1β, TNF-α and COX-2 protein, compared with levels in the vehicle group (Figures 4 and 5).

Examination of postmortem brains of AD patients reveals the abundant presence of inflammatory mediators, such as pro-inflammatory cytokines and chemokines, e.g., IL-1, IL-6, TNF-α, MIP-1b, complement activation products, and oxygen radicals\cite{37, 38, 39}. Inflammatory processes are present also in transgenic AD mouse models\cite{37, 38, 39}. Our results indicated that evodiamine decreased the levels of IL-1β (Figure 4). IL-1 is reported to induce expression of AChE protein and mRNA and to increase AChE enzyme activity, and that such an effect exacerbates cholinergic decline and dysfunction in AD\cite{40}. Our results also indicated that evodiamine decreased the levels of IL-6 (Figure 4). IL-6 occurs normally at barely detectable levels in the adult CNS, and is strongly induced under pathological conditions\cite{37, 38}. We found that evodiamine decreased the levels of TNF-α (Figure 4). TNF-α is a proinflammatory cytokine, the biological effects of which include stimulation of the acute-phase response, and cytotoxicity; furthermore, TNF-α stimulates IL-1 and IL-6 production, expression of adhesion molecules, and procoagulant activity\cite{41}. In our studies evodiamine was shown to decrease the levels of COX-2 protein (Figure 5).

COX-2 is an enzyme that plays a pivotal role in the arachidonic cascade leading to prostaglandin synthesis. Because the latter is so deeply intertwined with other inflammatory mechanisms, the inhibition of COX-2, with the attendant inhibition of prostaglandins, is a central target for anti-inflammatory therapy\cite{42}. COX-2 mRNA and protein are considerably up-regulated in affected areas of the brain in AD. COX-2 helps mediate production of prostaglandins and other inflammatory factors, and it is itself up-regulated by pro-inflammatory mediators. For example, IL-1 and TNF-α are known to regulate COX-2 expression. Thus, this enzyme occupies a pivotal amplifying position for inflammatory reactions\cite{43, 44}. Since evodiamine inhibits the expression of IL-1β, IL-6, TNF-α, and COX-2 protein, it may effect attenuation of CNS dysfunction in AD. However, the role of inflammation in the pathogenic process is still a matter of debate. One proposal is that the deposition of fibrillated Aβs in the human cortex could induce a local inflammatory reaction. Thus, neuro-inflammation is still considered to be a downstream consequence in the amyloid hypothesis, and inflammation has been considered as a secondary bystander response to neuronal degeneration and death\cite{45}. Salminen \textit{et al}\cite{46} indicate that increased production of amyloid-β oligomers can activate the innate immunity system via pattern-recognition receptors and evoke the pathology of AD. In addition, the pathology of AD seems to be the outcome of the activation of innate immunologic defenses in the brain. Therefore, inflammation is not merely a bystander in neuro-degeneration but a powerful pathogenic force in the disease process.

The preponderance of our present findings suggests that evodiamine could alleviate impairment of learning abilities and memory and significantly improve the glucose uptake in the APP\textsuperscript{swe}/PS1\textsuperscript{ΔE9} transgenic mice, and that the therapeutic effect of evodiamine was likely mediated through...
the inhibition of the inflammatory process, but not of senile plaque reduction. Our results suggest that evodiamine could have potential usage in treatment of AD.

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Author contribution
Lian-feng ZHANG designed research; Shu-min YUAN performed research; Kai GAO contributed PET/CT images analysis; Xiong-zhi QUAN helped with Western blot analysis; Jiang-ning LIU helped with modifying figures; Chuan QIN, Dong-mei WANG, Chun-mei MA contributed pathological analysis; and Lian-feng ZHANG and Shu-min YUAN wrote the paper.

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