In *Salvia miltiorrhiza*, phenolic acids possess protective properties against amyloid β-induced cytotoxicity, and tanshinones act as acetylcholinesterase inhibitors

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**Abstract**

*Radix Salvia miltiorrhiza* (RSM), a traditional Chinese medicinal herb, has been alleged to possess therapeutic effects against senile dementia, also known as Alzheimer’s disease (AD). However, the effects of the major components in RSM on cytotoxicity induced by amyloid-β peptide (Aβ) and on acetylcholinesterase activity have not been studied in depth to date. In this report, the effects of RSM aqueous/ethanol extracts, total polyphenols, total tanshinones and 3 phenolic compounds against toxicity mediated by Aβ25–35 were tested with PC-12 cells. The results showed that Aβ25–35-induced cytotoxicity was revised by RSM aqueous/ethanol extracts and total polyphenols and that danshensu and salvianolic acid B could protect PC-12 cells by blocking Aβ25–35-induced Ca2+-intake, lactate dehydrogenase release, cell viability decrease and apoptosis. In addition, the activities of RSM extracts and relevant constituents in their inhibition of acetylcholinesterase were investigated using rat brain homogenates as an enzyme resource. Galanthamine hydrobromide, an accepted acetylcholinesterase inhibitor, was employed as a positive control agent. Our preliminary studies demonstrated that RSM ethanol extract, total tanshinones, tanshinone I and dihydrotanshinone I had remarkable inhibition effects on acetylcholinesterase in vitro. These findings suggest that both tanshinones and polyphenols in RSM are the active constituents responsible for the beneficial effects of this herb in AD treatment.

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**Keywords:**
*Radix Salvia miltiorrhiza*, Amyloid β, Acetylcholinesterase

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**1. Introduction**

*Radix Salvia miltiorrhiza* (RSM), the root of *Salvia miltiorrhiza* Bunge, is one of the most widely used traditional herb medicines for the treatment of a variety of conditions, such as cardiovascular (Han J.Y. et al., 2008; Zhao et al., 2006b) and cerebrovascular diseases (Chan et al., 2004), hepatitis (Song et al., 2008; Wan et al., 2006), hepatocirrhosis (Yang et al., 2008; Liu et al., 2001), hepatoma (Lin et al., 2000) and chronic renal failure (Kang et al., 2004). Chemically, the active constituents of RSM can be classified into two groups. The water-soluble com-
ponents are phenolic compounds consisting of danshensu, protocatechuic aldehyde, salvianolic acid B, etc. (Gu et al., 2007; Liu et al., 2006). The lipid-soluble components are mainly tanshinones such as tanshinone I, tanshinone IIA, dihydrotanshinone I and cryptotanshinone, which belongs to a group of diterpenes with an abietane-type skeleton (Don et al., 2006; Shi et al., 2005). In recent years, the water-soluble polyphenols have attracted attention increasingly because of their effectiveness in inhibiting platelet aggregation (Tang et al., 2002) and as antioxidants for the removal of free radicals, thus potentially treating atherogenic dyslipidemia (Ji and Gong, 2008) and cholestatic liver injury (Oh et al., 2002). As for lipid-soluble tanshinones, it is reported that they can modulate mutagenic activity (Kang et al., 2000; Wu et al., 2007a,b), inhibit cancer cell proliferation (Mosaddik, 2003; Yoon et al., 1999), protect hepatocytes from bile acid-induced apoptosis (Park et al., 2007), and control excessive alcohol consumption in alcoholics (Brunetti et al., 2003; Colombo et al., 1999).

Recently, the neuroprotective potential of salvianolic acid B against amyloid β peptides (Aβ) induced cytotoxicity in the rat pheochromocytoma line PC-12 cells has been explored. Salvianolic acid B, the major phenolic component in RSM, can protect against Aβ25-35-induced reduction in expression of brain–pancreas relative protein through its effects on suppressing the production of ROS, calcium influx, and apoptosis (Lin et al., 2006; Durairajan et al., 2008). Consideration of the neuroprotective properties of salvianolic acid B prompts us to further investigate whether there are other active ingredients in RSM. For this purpose, the effects of RSM aqueous/ethanol extracts, total polyphenols, total tanshinones and 3 phenolic compounds against cytotoxicity mediated by Aβ25-35 were tested with PC-12 cells. The MTT reduction assay, extracellular lactate dehydrogenase and intracellular Ca2+ determinations, as well as apoptosis staining were used to quantitatively or qualitatively evaluate cell viability and injury. In addition, we investigated the activities of RSM aqueous/ethanol extracts, total polyphenols, total tanshinones and 4 tanshinone compounds in their inhibition of acetylcholinesterase using rat brain homogenates.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (∼200 g), provided by the Academy of Military Medical Sciences (Beijing, China), were used in this study. Animals were housed in stainless steel cages in a room maintained in a 12 h light/dark cycle at around 22 °C. Food and water were available ad libitum. Animals were allowed to aclimatize for at least 5 days prior to the experiment. All animal care and treatment were conducted in conformity with the NIH Guide for the Care and Use of Laboratory Animals, and in accordance with the Animal Ethics Committee of China Pharmaceutical University.

2.2. Chemicals and other reagents

Radix Salvia miltiorrhiza, provided by Shanxi Tasy Plants Pharmaceutical Co. Ltd., Shanxi Province, China, were authenticated by Professor Li Ping, Department of Traditional Chinese Medicine, China Pharmaceutical University. Amyloid β-protein fragment 25–35 (Aβ25-35), bisbenzimide H 33258 (HO33258), propidium iodide (PI), Fura 2-AM and galanin analogue hydrobromide (purity ≥ 94%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Stock Aβ25-35 solution was prepared by dissolving the peptide in sterile tri-distilled water at a concentration of 75 μM and was kept at −20 °C for future use. RPMI 1640-based culture medium and new-born calf serum were bought from HyClone laboratories, Inc. (UT, USA). The reagent kits for the activity measurement of lactate dehydrogenase (LDH) and acetylcholinesterase (AChE) was obtained from Jiancheng Bioengineering Institute (Nanjing, China). Dihydrotanshinone I (purity ≥ 98%) was purchased from Winherb Medical Science Co. Ltd. (Shanghai, China). Danshensu and other pure compounds (protocatechuic aldehyde, salvianolic acid B, tanshinone I, tanshinone IIA, and cryptotanshinone) were collected from Chinese National Institute for the control of pharmaceutical and biological products (Beijing, China), and their purities were all above 98%. HPD400 macroporous resin was bought from Bonchem Co. Ltd. (Cangzhou, China).

2.3. Preparation of sample solutions RSM aqueous/ethanol extracts

The fine powder (30-mesh size) of RSM was extracted twice with ten volumes of boiled water under refluxing for 1 h. The combined physic liquor, after concentrated under reduced pressure, were filtered, condensed in RSM aqueous/ethanol extracts, total polyphenols, total tanshinones and 4 tanshinone compounds in their inhibition of acetylcholinesterase using rat brain homogenates.

The fine powder (30-mesh size) of RSM was extracted twice with ten volumes of boiled water under refluxing for 1 h. The combined physic liquor, after concentrated under reduced pressure, were filtered, condensed in RSM aqueous/ethanol extracts, total polyphenols, total tanshinones and 4 tanshinone compounds in their inhibition of acetylcholinesterase using rat brain homogenates.

The fine powder (30-mesh size) of RSM was extracted twice with ten volumes of boiled water under refluxing for 1 h. The combined physic liquor, after concentrated under reduced pressure, were filtered, condensed in RSM aqueous/ethanol extracts, total polyphenols, total tanshinones and 4 tanshinone compounds in their inhibition of acetylcholinesterase using rat brain homogenates.

The fine powder (30-mesh size) of RSM was extracted twice with ten volumes of boiled water under refluxing for 1 h. The combined physic liquor, after concentrated under reduced pressure, were filtered, condensed in RSM aqueous/ethanol extracts, total polyphenols, total tanshinones and 4 tanshinone compounds in their inhibition of acetylcholinesterase using rat brain homogenates.
and 1.27%, respectively. These two constituent fractions were dissolved in 2% DMSO and filtered with a sterile 0.2-μm filter before administration.

2.5. Monomeric components (3 polyphenols and 4 tanshinnones)

Danshensu, protocatechuic aldehyde and salvianolic acid B were directly dissolved in tri-distilled water because of good water-solubility. However, galanthamine hydrobromide, tanshinone I, tanshinone IIA, dihydrotanshinone I and cryptotanshinone were dissolved in 2% DMSO due to their low polarity. The various kinds of standard substance solutions were filtered with sterile 0.2-μm filter before administration. The purity of galanthamine hydrobromide was above 94%, and the purities of other constituents were all above 98%.

2.6. Cell culture

Rat pheochromocytoma PC-12 cell line was maintained in culture flask in RPMI 1640-based medium supplemented with 10% new-born calf serum. Cell cultures were maintained at 37°C in an atmosphere of 95% air/5% CO2 saturated with H2O. For MTT assay and LDH determination, cells were seeded into 96-well microplates (Corning Incorporated, USA) at a density of 10^3 cells/ml. For intracellular Ca2+ measurement and HO33258/PI staining, cells were plated onto 24-well plates at a density of 4 x 10^4 and 10^5 cells per well, respectively. All experiments were carried out 24 h after the cells were seeded.

2.7. Cell viability assay

Cell viability was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay. After seeding in 96-well microplates for 24 h, cells were incubated for 48 h with 15 μM Aβ25-35 in the absence or presence of drug solutions as described in figure legends. Cellular viability was evaluated by the reduction of MTT to formazan. Briefly, 4 h after incubated with 0.5% MTT (20 μl) at 37°C, cells were lysed in 150 μl DMSO and the optical density at 570 nm (OD570 nm) was determined in a Microplate Reader (Dynatec Laboratories, Alexandria, VA, USA). All values were presented as means ± S.D. of numbers obtained from six wells and three separate experiments.

2.8. Measurement of intracellular Ca2+

The concentration of intracellular Ca2+, [Ca2+]i, was monitored by the method of Fura 2-AM double wavelength fluorescence scanning as described previously (Berts and Minneman, 2000). The attached monolayer cells were trypsinized and preloaded with Fura 2-AM (10 μM) for 50 min at 37°C in the dark. After loading with Fura 2-AM, cells were collected and gently washed twice with HBSS, then resuspended in HBSS solution containing 0.2% BSA. The intracellular [Ca2+]i was measured with a fluorospectrophotometer (Shimadzu RF-5301, Japan) at 340 nm and 380 nm. [Ca2+]i was calculated with Kd of 224 nM by applying the following formula: [Ca2+]i = Kd × (R - Rmin) (Sf/Sf0) / (Rmax - R) in which R indicates the rate of F340 nm/F380 nm, and Sf0 and Sf indicate the fluorescence intensity at 380 nm of [Ca2+]i = 0 and Ca2+ saturation, respectively. Data were directly expressed as the concentrations of intracellular Ca2+. All values were presented as means ± S.D. of numbers obtained from three wells and three independent experiments.

2.9. Assessment of LDH leakage

Extracellular LDH, released from damaged cells, was an indicator of cell injury. In this study, PC-12 cell injury was quantitatively evaluated by the determination of LDH with a reagent kit. Its mechanism was that through catalyzed action of LDH, lactate could convert to pyruvate, which would react with dinitro-phenylhydrazine and produce the pyruvate – dinitrophenylhydrazone, whose colour was red in alkaline solution. The value of chromaticity was proportional to LDH activity in the sample. Briefly, after cells were incubated with Aβ25-35 in the absence or presence of drug solutions for 48 h, cell-free culture supernatants were then gently aspirated and collected for LDH determination according to the manufacturer's protocol. Then the intensity of the red colour was measured at 450 nm in a microplate reader. All data were presented as means ± S.D. of numbers obtained from six wells and three separate experiments.

2.10. HO33258/phosphotydyl inositol (PI) double staining

Normal cells and apoptosis cells can be stained by HO33258. However, they cannot be stained by PI on account of their intact cell membranes. Dead cells can be stained by PI because of their increasing cell membrane permeability. Under a fluorescence microscope (Carl Zeiss Shanghai Co., Ltd. NO, MIC 00094), normal cells present aequalis blue fluorescence; dead cells show red fluorescence; apoptotic cells give uneven blue fluorescence with other effect such as chromatin condensation, cell crenulation and frothing, as well as nuclear fragmentation. After incubated for 48 h with Aβ25-35 and drugs, the attached cells were stained by 150 μl HO33258 (10 mg/ml) and 150 μl PI (15 mg/ml) for 30 min at 4°C followed by taking pictures promptly (Joshua et al., 2007).

2.11. Assay for acetylcholinesterase activity

Acetylcholinesterase activity assay was carried out using a reagent kit based on the colorimetric method as described previously (Kim et al., 2006), with several differences. The male Sprague–Dawley rats (~200 g) were acclimatized to the facilities for 5 days, and then fasted, free access to water for 12 h prior to experiment. Brain samples were obtained after the animals were decapitated and then the samples were frozen at –20°C until analysis. Whole brain was homogenized using a hand-held laboratory homogenizer (Ginotech pro-200, China) with nine volumes of physiological saline, and then centrifuged at 3500 rpm for 10 min. The 10% brain homogenate was used as enzyme source for acetylcholinesterase inhibition assay. Briefly, after incubated with or without drug solutions at 37°C for 30 min, brain homogenate was aspirated and collected for enzyme activity determination using an assay kit. The optical density at 412 nm (OD412 nm) was determined in a
Fig. 1 – Protective effects of RSM extracts and fractions on Aβ25-35-mediated cytotoxicity in PC-12 cells. PC-12 cells were incubated with 15 μM Aβ25-35 in the absence or presence of drugs. After this incubation, cell viability was determined with the MTT assay. (A) PC-12 cells were treated with RSM aqueous/ethanol extracts (0.001–2 mg/ml); (B) PC-12 cells were treated with RSM polyphenols/tanshinones fractions (0.01–100 μg/ml). Values were represented as means ± S.D. (n = 6). *Significantly different from normal group; significantly different from Aβ25-35 treated group (*p < 0.05, **p < 0.01).

Fig. 2 – Protective effects of 3 phenolic compounds in RSM against Aβ25-35-induced PC-12 cell death. PC-12 cells were incubated with 15 μM Aβ25-35 in the absence or presence of drugs. After this incubation, cell viability was determined with the MTT assay. Values were represented as means ± S.D. (n = 6). *Significantly different from normal group; significantly different from Aβ25-35 treated group (*p < 0.05, **p < 0.01).

Considering the mainly containing constituents of RSM, we further investigated the protective effects of RSM polyphenols/tanshinones fractions on Aβ25-35-induced cell death. Fig. 1B shows that the active components of RSM resided in the polyphenols fraction. At 1–100 μg/ml, the polyphenols fraction significantly protected PC-12 cells from the toxicity of Aβ25-35, increasing the cell viability to 11–53% compared with Aβ-treated group. Under the same concentration range, RSM tanshinones fraction did not exhibit any cytoprotective effect, unexpectedly, showed significant cytotoxicity at high concentrations (10–100 μg/ml). Two possible explanations, we concluded, may contribute to the cytotoxic effect of tanshinones fraction at 10 μg/ml or higher. One possible explanation was that excessive dosage of the tanshinone fraction could result in pH change of cell medium that was unfavorable for cells survival. The other possible explanation was that the traces of cytotoxic substances accumulated continuously along with the increasing dosage of tanshinone fraction. However, these hypotheses may require further investigation.

3.2. The cytoprotective activity of phenolic compounds in RSM

To identify the cytoprotective components of RSM polyphenols fraction, 3 pure phenolic compounds were collected and tested in PC-12 cells by MTT assay. The 3 compounds were important phenolic constituents in RSM, including protocatechuic aldehyde, danshensu, salvianolic acid B. As shown in Fig. 2, 15 μM Aβ25-35 significantly decreased the cell viability, however, the cytotoxic effects were attenuated by the co-treatment with danshensu or salvianolic acid B. At 10–200 μg/ml, danshensu and salvianolic acid B all markedly blocked cytotoxic effects of Aβ25-35 on viability. Unlike danshensu and salvianolic acid B, protocatechuic aldehyde showed no protective effect against cytotoxicity of Aβ25-35 at the same concentrations tested.
ever, Aβ25–35-induced Ca2+-intake in PC-12 cells. After seeded in 96-well plates at 10^5/ml for 24 h, cells were incubated with Aβ25–35 or medicines for 48 h. Cell-free culture supernatants were collected and treated with the appropriate reagent mixture. A colorimetric assay was applied for determining LDH release. Values were represented as means ± S.D. (n = 6). *Significantly different from normal group; significantly different from Aβ25–35 treated group (p < 0.05, **p < 0.01).

3.3. Effects of danshensu and salvianolic acid B on Ca2+-intake in PC-12 cells

The neurotoxic effect of Aβ25–35 was relevant to inducing influx of Ca2+ from the extra-cellular space into the cell body through the L-type voltage-dependent Ca2+-channel. Hence, danshensu and salvianolic acid B might have acted by blocking Aβ-induced Ca2+-intake in PC-12 cells. To test this hypothesis, we investigated whether danshensu and salvianolic acid B could attenuate Aβ25–35-induced increase in intracellular calcium concentration. Exposure of PC-12 cells to 15 μM Aβ25–35 for 48 h led to a 1.4-fold increase of [Ca2+]i shown in Fig. 3. However, Aβ25–35-induced cytosolic Ca2+ influx was suppressed markedly in the cells incubated with danshensu or salvianolic acid B. At 200 μg/ml, danshensu and salvianolic acid B could restore the [Ca2+]i elevation for 93.78% and 81.28%, respectively.

3.4. Effects of danshensu and salvianolic acid B on LDH release in PC-12 cells

Automated quantification, as opposed to morphological assessment, of cell death can involve assays for determining loss of plasma membrane integrity or measurements of a biochemical perturbation. One method of assaying loss of membrane integrity was measuring release into the bathing medium of the cytosolic enzyme, LDH. In this study, the quantitative assessment of LDH in extracellular fluid was performed to further investigate the protective effects of danshensu and salvianolic acid B. Fig. 4 shows that 15 μM Aβ25–35 caused about 40% additional LDH release from Aβ-treated cells as compared with normal cells. The LDH release mediated by Aβ25–35 was significantly inhibited by the co-treatment with danshensu or salvianolic acid B. At 100 μg/ml, danshensu and salvianolic acid B could decrease the LDH release from PC-12 cells to 78% and 75%, respectively. The protective effects of danshensu and salvianolic acid B on Aβ25–35-induced cytotoxicity determined by LDH assay were similar to that determined by MTT assay.

3.5. Danshensu and salvianolic acid B rescue Aβ25–35-induced morphological changes in PC-12 cells

HO33258/PI double staining were additionally employed to visualize apoptotic changes and cell damage. As shown in Fig. 5B, PC-12 cells incubated with Aβ25–35 appeared morphological changes including obvious nuclear aggregation, karyoclasis and cell crenulation, and moreover, some dead cells were stained by PI and emitted red fluorescence because of their enhanced membrane permeability. However, these changes in nuclear characteristics of apoptosis were alleviated markedly in the cells co-incubated with 200 mg/ml danshensu or salvianolic acid B (Fig. 5C and D).

3.6. The acetylcholinesterase inhibition of RSM resides in its tanshinones fraction

In order to explore the acetylcholinesterase inhibition of RSM, the aqueous extract and the ethanol extract were tested using rat brain homogenates as enzyme resource. As shown in Fig. 6A, all of the various concentrations of RSM ethanol extract could significantly block acetylcholinesterase activity in vitro. Meanwhile, ethanol extract at the highest concentration (2 mg/ml) caused the enzyme activity decreasing to 73%. For RSM aqueous extract, only 1 mg/ml extract slightly inhibited acetylcholinesterase activity at the same concentration range tested.

To identify the category of active constituents, RSM polyphenols and tanshinones fractions were prepared and tested for their activities of acetylcholinesterase inhibition.
Fig. 5 – Effects of danshensu and salvianolic acid B on Aβ$_{25-35}$-induced apoptotic neurotoxicity in PC-12 cells. After incubated for 48 h, the cellular media were removed by aspiration. The attached cells were stained by 150 μl HO33258 (10 mg/ml) and 150 μl PI (15 mg/ml) for 30 min at 4°C followed by taking pictures promptly (magnification, 200 ×). (A) Normal PC-12 cells stained by HO/PI; (B) PC-12 cells stained by HO/PI after incubated with 15 μM Aβ$_{25-35}$ for 48 h; dead cells show red fluorescence; apoptotic cells yield uneven blue fluorescence including some symptoms such as chromatin aggregation, caryoclasis, cell crenulation and frothing; (C) PC-12 cells stained by HO/PI after incubated with 15 μM Aβ$_{25-35}$ and 200 μg/ml danshensu; (D) PC-12 cells stained by HO/PI after incubated with 15 μM Aβ$_{25-35}$ and 200 μg/ml salvianolic acid B. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

**3.7. The acetylcholinesterase inhibition of tanshinones compounds in RSM**

To confirm the indeed active components of RSM tanshinones fraction, 4 pure tanshinones compounds were collected and tested for their acetylcholinesterase inhibition in vitro. The 4 compounds were important tanshinones constituents in RSM, including tanshinone I, tanshinone IIA, dihydrotanshinone I and cryptotanshinone. In addition, galanthamine hydrobromide was used as a positive control. As shown in Fig. 7, tanshinone I (1–100 μM) and dihydrotanshinone I (0.01–100 μM), as well as galanthamine hydrobromide (0.01–10 μM) significantly inhibited acetylcholinesterase activity. Meanwhile, neither tanshinone IIA nor cryptotanshinone exhibited any acetylcholinesterase inhibition effect at the same concentration range. Moreover, the IC$_{50}$ value of galanthamine hydrobromide was calculated as 0.22 μM, which was similar to previously published data (Thomsen and Kewitz, 1990). Tanshinone I and dihydrotanshinone I did not reveal a dose–response relationship, hence, their IC$_{50}$ values were not provided.

**4. Discussion**

Alzheimer’s disease (AD) is one of the most common neurodegenerative disorders associated with aging, which is characterized by fibrillar deposits of amyloid β peptides in the cerebral neocortex and hippocampus (Andreasen and Blennow, 2002; Harigaya et al., 2000). The deposition of amyloid β peptides is a pathological hallmark of AD and is a key event in the pathogenesis of AD (Iwatsubo, 1998; Selkoe, 2001). Aβ, which is normally secreted from amyloid precursor protein (APP), is usually proteolytically degraded within a short period. However, upregulation of the secretion of Aβ from APP, or an increased ratio of Aβ may render the Aβ liable to be retained in the brain (Kawahara and Kuroda, 2000). In cultures, Aβ can directly induce neuronal cell death and can render neurons vulnerable to excitotoxicity and oxidative insults (Hardy, 1997). The mechanisms underlying β-amyloid peptide neurotoxicity are complex but may involve disruption of intracellular homeostasis of Ca$^{2+}$ and potassium, induction of oxidative stress as well as upregulation of apoptosis.
Fig. 6 – Inhibitory effects of RSM extracts and fractions on acetylcholinesterase activity in rat brain homogenates. After incubated with or without drugs for 30 min at 37 °C in vitro, the enzyme activities of brain homogenates were determined according to an assay kit. The value for each treatment group was converted to the percentage of the untreated control group. (A) RSM aqueous/ethanol extracts (0.001–2 mg/ml) were administered; (B) RSM polyphenols/tanshinones fractions (0.1–1000 μg/ml) were administered. Data were expressed as means ± S.D. (n = 3). Significantly different from the control group (*p < 0.05, **p < 0.01).

Fig. 7 – Inhibitory effects of 4 tanshinones compounds in RSM and galanthamine on acetylcholinesterase activity in rat brain homogenates. After incubated with or without drugs for 30 min at 37 °C in vitro, the enzyme activities of brain homogenates were determined according to an assay kit. The value for each treatment group was converted to the percentage of the untreated control group. Data were expressed as means ± S.D. (n = 3). Significantly different from the control group (*p < 0.05, **p < 0.01).
that the carboxyl of phenylpropionic acid unit was crucial for the protective activities of phenolic compounds. However, the exact mechanisms of the structure–function relationship with these agents remain to be elucidated.

Among the various approaches applied to the treatment of AD, acetylcholinesterase inhibitors as a class have been the most clinically successful in ameliorating cognitive dysfunction. Drugs such as tacrine, donepezil and galantamine were proven effective in delaying the progression of the disease, at the expense of several adverse reactions (Villarroya et al., 2004; Colombres et al., 2004). This suggests that natural AChEIs isolated from plant sources may have potential efficacy, which is advantageous in the light of their low adverse effects and prices. Recent studies have shown that tanshinone congeners significantly reversed scopolamine-induced cognitive impairments and that cryptotanshinone and dihydrotanshinone I inhibited acetylcholinesterase activities in vitro and ex vivo (Kim et al., 2007). In this present study, we systematically investigated the activities of RSM aqueous/ethanol extracts, total polyphenols, total tanshinones and 4 tanshinone compounds in their inhibition of acetylcholinesterase. Our results indicate that RSM ethanol extract, total tanshinones, and 2 tanshinone compounds have anti-acetylcholinesterase activities in vitro. Unlike previous reports (Kim et al., 2007; Ren et al., 2004), tanshinone I and dihydrotanshinone I were found to be the major active components in tanshinone compounds in our research. As shown in Fig. 8, tanshinone I and dihydrotanshinone I are similar in their structure with common aromatic A rings, except that the former contains a double bond at C15 and C16 in the furan ring. However, tanshinone IIA and cryptotanshinone are different in furan ring and same in cyclic A ring. The present results suggested that the aromatic A ring was the factor principal for acetylcholinesterase inhibitory activity of tanshinone congeners, whereas the furan ring was less important for acetylcholinesterase inhibition, although this requires further evaluation.

In conclusion, the present study demonstrates that both tanshinones and polyphenols in Radix Salvia miltiorrhiza may possess therapeutic effects against AD. On one hand, the polyphenolic compounds represented by danshensu and salvianolic acid B have protective properties against Aβ25–35-induced neurotoxicity. On the other hand, the tanshinone constituents, such as tanshinone I and dihydrotanshinone I, possess anti-acetylcholinesterase activity in vitro. The potential relevance of the present results to clinical application in the treatment of AD will be investigated in future studies.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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