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Multi-Target Strategy and Experimental Studies of Traditional Chinese Medicine for Alzheimer's Disease Therapy

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Abstract: Alzheimer's disease (AD) is a multifactorial complex disease. The pathogenesis of AD is very complicated, and involves the β-amyloid (Aβ) cascade, tau hyperphosphorylation, neuroinflammation, oxidative stress, mitochondrial dysfunction, reduced levels of neurotrophic factors, and damage and loss of synapses as well as cholinergic neurons. The multi-target characteristics of traditional Chinese medicine (TCM) may be advantageous over single-target drugs in the treatment of complex diseases. These drugs have therefore attracted more attention in the research and development of AD therapies. This review describes advances made in experimental studies of TCM for AD treatment. It discusses research, from our group and other laboratories, on TCM compound drugs (Shenwu capsule) and approximately 10 Chinese medicinal herb extracts (tetrahydroxystilbene glucoside, epimedium flavonoid, icariin, cornel iridoid glycoside, ginsenoside, puerarin, clausenamide, huperzine A, and timosaponins).

Keywords: Alzheimer's disease, Animal model, Compound drug, Herb extract, Multi-target, Therapy, Traditional Chinese medicine.

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive cognitive impairment and is the most common type of dementia found in the aging population. Three significant pathological changes are observed in the brain of patients with AD: senile (neuritic) plaques caused by the deposition of β-amyloid (Aβ), neurofibrillary tangles caused by hyperphosphorylation of the tau protein, and a severe loss of cholinergic neurons. The etiology of AD is unclear; AD pathogenesis is very complicated and multiple factors are involved in AD progression, including the Aβ cascade, tau hyperphosphorylation, neuroinflammation, oxidative stress, neurotrophic factor reduction, synapse loss, and cholinergic neuron death [1]. In addition, aging, diabetes, cerebral ischemia, and trauma are risk factors for AD [2]. Single target effects of new drugs may cause them to fail in AD clinical trials. Therefore, many investigators have proposed the need for development of multi-target approaches for AD therapy [1]. To treat complex diseases, the multi-target characteristics of compounds used in traditional Chinese medicine (TCM) may have advantages over single-target drugs; therefore, TCM is attracting more attention in AD therapy research and development. In this review, we describe the advances in research, from our group and other laboratories, on TCM compound drugs and herb extracts which act on multi-targets in pathogenesis of AD, for the purpose of providing novel strategies for AD therapy.

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2. SHENWU CAPSULE-A COMPOUND TCM DRUG

According to the theory of TCM, AD pathogenesis mainly includes deficiencies of the kidney and spleen, phlegm turbidity, and blood stasis. Based on extensive literature searches and results of our screening experiment in combination with TCM theory and clinical experience, we created a new TCM compound drug called Shenwu capsule (code name: 962 capsule) for the treatment of AD in 1996. Shenwu capsule (SW) consists of six traditional Chinese herbs, including *Polygonum multiflorum, Ginseng,* and *Pueraria*. Shenwu tonifies the kidney and spleen and eliminates phlegm and blood stasis, which matches the therapeutic principle of TCM for AD. Because of the complicated pathogenesis of AD, we used a variety of AD-like animal models to determine the pharmacological effects and explore the mechanisms of SW for the treatment of AD.

2.1. Effects on Cholinergic Damage-Induced AD Model

Consistent observations in the brains of patients with AD include a considerable loss of cholinergic neurons and decrease in the ratio of choline acetyltransferase (ChAT)/acetylcholine esterase (AChE). AD-like model rats were generated by administering an injection of ibotenic acid (an excitatory amino acid) into the basal forebrain [3] or an intraperitoneal injection of scopolamine (an anticholinergic agent) [4]. Oral administration of SW improved learning and memory impairments, increased the ChAT/AChE ratio, and enhanced muscarinic cholinergic receptor binding in the cerebral cortex and hippocampus of these model rats [3, 4].

2.2. Effects on Aβ Cascade

The Aβ cascade is one of the core theories of the pathogenesis of AD. Using APPV717I transgenic mice, we found

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that SW treatment ameliorated learning and memory deficits, decreased the number of amyloid plaques, Aβ content, and the expression of β-secretase and presenilin 1 (PS1), and reduced the expression of α-synuclein mRNA and protein in the hippocampus and cerebral cortex [5-7]. Further, we discovered that SW reduced neuronal damage and loss, inhibited the activation of microglia and astrocytes, decreased the levels of proinflammatory cytokines including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), and suppressed nitric oxide synthase (NOS) activity and nitric oxide (NO) content in the hippocampus of rats in which Aβ25-35 was injected into the hippocampus [8].

2.3. Effects on Mitochondrial Deficiency-Induced AD Model

Levels of mitochondrial cytochrome c oxidase (respiratory chain complex IV) are reportedly decreased in the AD brain. To establish an AD rat model, we used a subcutaneous micro-pump to continuously infuse sodium azide, a complex IV inhibitor. In this model, the learning and memory ability was decreased, ChAT activity and brain-derived neurotrophic factor (BDNF) expression were reduced, the levels of amyloid precursor protein (APP), Aβ, β-secretase, PS1, phosphorylated tau protein, and glycogen synthase kinase-3β (GSK-3β) were increased, while protein phosphatase 2A (PP2A) was decreased in the hippocampus and cerebral cortex, as compared with the sham control rats. These findings suggest that mitochondrial dysfunction may be an early initiating factor in AD. In the experiment using sodium azide-treated rats, we found that SW: (1) improved learning and memory impairment, (2) increased the ChAT/AChE ratio and muscarinic cholinergic receptor binding, (3) enhanced the expression of BDNF and its receptor TrkB, (4) decreased the levels of APP, Aβ, β-secretase, and PS1, and (5) reduced phosphorylated tau protein, and increased PP2A content in the hippocampus and cerebral cortex. These data suggest that SW may exert its effects during the early stage of AD [9-11].

2.4. Effects on Aging Model

Aging is the greatest risk factor for AD. Various changes in the brains of aging rats are similar to the pathological changes observed in humans with AD, and can simulate the characteristics of chronic neurodegeneration in AD. In our experiments using aged rats (24-months-old), SW enhanced cognitive function, increased the expression of synaptophysin, nerve growth factor (NGF) and its receptor TrkA, elevated the number of ChAT-positive cholinergic neurons, and inhibited the activation of glial cells in the hippocampus [12-16]. In the spinal cord of aged rats, SW treatment also increased the expression of NGF, BDNF, glial-derived neurotrophic factor (GDNF), and phosphorylated cAMP responsive element binding protein (p-CREB), reduced Bax expression, and enhanced Bcl-2 expression [17, 18].

Oxidative stress plays an important role in the pathogenesis of AD. In our experiments using a brain-aging mouse model in which mice were subjected to subcutaneous D-galactose injection, SW treatment improved learning and memory impairment, and decreased the lipid peroxide content in the brain [19].

2.5. Effects on Cerebral Ischemia-Induced Dementia Model

Cerebral ischemia is an important factor in promoting the development of AD. In our experiments using a chronic cerebral hypoperfusion rat model, which was induced by permanent bilateral ligation of the common carotid artery, SW treatment ameliorated cognitive impairment, reduced the damage and loss of neurons and the activation of astrocytes, and enhanced the expression of neurotrophic factor-3 (NT-3) in the hippocampus of model rats [20-22].

2.6. Effects on Diabetes-Induced Dementia Model

Diabetes is a risk factor for AD, and patients with diabetes usually present with vascular lesions. We established a complex rat model by intraperitoneal injection of streptozotocin followed by bilateral common carotid artery occlusion and reperfusion. SW treatment improved learning and memory, increased long-term potentiation (LTP) in the hippocampus (indicating enhanced synaptic plasticity), elevated the expression of NT-3 and its receptor TrkC, increased the expression of Akt in the neuronal survival signaling pathway, up-regulated Bcl-2 and down-regulated Bax and caspase-3 expression, and reduced neuronal damage and loss in the hippocampus of model rats [23-25].

2.7. Summary

These results demonstrate that SW, a new compound TCM drug, improves learning and memory defects in a variety of AD-like animal models. Further, SW prevents or delays neuronal degeneration and death due to its beneficial effects on multiple targets in the complicated pathogenesis of AD, including enhancing cholinergic function, inhibiting the Aβ cascade, tau hyperphosphorylation and inflammation, increasing neurotrophic factors, and protecting synapses and mitochondria. Completed phase III clinical trial of SW in China demonstrated that SW treatment effectively improved cognitive impairment in patients with mild to moderate AD. These results suggest that a multi-target strategy may be beneficial in AD therapy.

3. POLYGONUM MULTIFLORUM EXTRACT

Polygonum multiflorum (Chinese name: Heshouwu) is one of the most popular Chinese medicinal herbs. According to TCM theory, processed *Polygonum multiflorum* is used to tonify the liver and kidney, and nourish the vital essence and blood. 2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside (TSG) is the major active component extracted from the tuberous roots of *Polygonum multiflorum*. We have investigated the effects of TSG on AD since 2002, and recently developed a new drug for the treatment of AD.

3.1. Effects on Cholinergic Damage-Induced AD Model

To explore the effects of TSG on cholinergic damage, we generated an AD-like animal model by injecting ibotenic acid into the basal forebrain of rats. We found that oral administration of TSG improved learning and memory impairments. Further, TSG treatment increased ChAT activity and the binding capacity of muscarinic cholinergic

receptors in the hippocampus and cerebral cortex of model rats [26, 27].

3.2. Effects on Aβ Cascade

In experiments using APPV717I transgenic mice, we found that TSG treatment enhanced learning and memory. TSG also reduced the number of amyloid plaques, Aβ content, and PS1 expression, inhibited α-synuclein expression and aggregation, and regulated phosphorylated extracellular signal-regulated kinase 1/2 (ERK1/2) in the hippocampus and cerebral cortex of model mice [28-32]. In addition, TSG ameliorated learning and memory deficits in mice that received an intracerebroventricular injection of Aβ1-40, and decreased the levels of IL-1β and IL-6, reduced the cyclooxygenase-2 (COX-2) and lipid peroxide content, and increased the total antioxidant capacity in the hippocampus and cerebral cortex [33-35]. In a hypercholesterolemia rat model, TSG improved learning and memory, reduced Aβ content in the hippocampus, and decreased the levels of serum cholesterol and low-density lipoprotein (LDL) [36, 37].

3.3. Effects on Mitochondrial Deficiency-Induced AD Model

In a rat model of AD, which was induced by subcutaneous mini-pump infusion of sodium azide, a mitochondrial complex IV inhibitor, we found that TSG treatment elevated the activity of mitochondrial cytochrome c oxidase (complex IV), enhanced the expression of NGF and BDNF, decreased Aβ, β-secretase, and PS1, and inhibited tau hyperphosphorylation and GSK-3β expression in the hippocampus of the model rats [38]. Preincubation of sodium azide-treated human neuroblastoma SH-SY5Y cells with TSG increased the mitochondrial membrane potential and adenosine triphosphate (ATP) content, decreased reactive oxygen species (ROS), and inhibited apoptosis via reducing Bax and enhancing Bcl-2 expression [38].

3.4. Effects on Aging Model

Our results showed that TSG ameliorated learning and memory dysfunction, protected the synaptic ultrastructure, increased the number of synapses and the expression of synaptophysin, elevated the expression of NGF and TrkA, and inhibited the loss of cholinergic neurons in the hippocampus of aged rats [16, 39]. TSG also elevated phosphorylation of calcium-calmodulin-dependent protein kinase II (CaMKII) which is involved in synaptic plasticity, and enhanced the expression of synaptophysin and postsynaptic density protein 95 (PSD95), as well as inhibited α-synuclein overexpression and aggregation in the hippocampus, striatum and cerebral cortex of aged mice.

In the brain-aging mouse model induced by subcutaneous injection of D-galactose, TSG improved learning and memory, increased glutathione (GSH) level and superoxide dismutase (SOD) activity, reduced the lipid peroxide content, enhanced the expression of NGF and TrkA, elevated the mRNA expression of a variety of energy metabolism enzymes, and attenuated the mRNA expression of inflammatory factors in the cerebral cortex and hippocampus of model mice [40-43].

3.5. Effects on Cerebral Ischemia-Induced Dementia Model

In a chronic cerebral ischemia rat model induced by permanent bilateral common carotid artery ligation, we found that TSG treatment ameliorated learning and memory deficits, mitigated neuronal damage and loss, increased the activity of GSH peroxidase, decreased the lipid peroxide level, and enhanced the expression of PP2A and microtubule-associated protein-2 (MAP-2) in the hippocampus and cerebral cortex of model rats [44, 45]. In acute cerebral ischemia models, TSG treatment increased SOD activity, reduced lipid peroxide content and intracellular Ca^{2+} overload in the brain of mice [46], decreased the binding capacity of N-methyl-D-aspartic acid (NMDA) receptors [47] in the forebrain of gerbils [48], and inhibited neuronal apoptosis in the brain of rats [49]. *In vitro* experiments showed that TSG treatment increased cell viability, decreased the leakage of lactate dehydrogenase (LDH), and inhibited intracellular Ca^{2+} overload induced by glutamate [50].

3.6. Effects on α-Synuclein and Parkinson's Disease Model

We discovered that TSG antagonized α-synuclein overexpression in the hippocampus of APP transgenic mice [32], an AD model. α-Synuclein also plays an important role in the pathogenesis of Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Therefore, we investigated the effects of TSG on PD dementia (PDD) and DLB. Following TSG treatment of α-synuclein-transfected PC12 cells, we found decreased α-synuclein mRNA and protein expression, inhibited α-synuclein aggregation, and enhanced expression of Parkin and ubiquitin C-terminal hydrolase-L1 (UCH-L1) in the ubiquitin-proteasome system [51]. TSG protected SH-SY5Y nerve cells against 1-methyl-4-phenylpyridinium (MPP^+)-induced cytotoxicity and apoptosis by increasing mitochondrial function, attenuating the accumulation of intracellular ROS, and decreasing the ratio of Bax/Bcl-2 and caspase-3 activation [52]. Another study examined mice in which PD-like symptoms were induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injection. Oral administration of TSG ameliorated the movement disorder, increased the number of tyrosine hydroxylase (TH)-positive neurons in the substantia nigra, and elevated the dopamine content in the striatum of the PD model mice [53].

3.7. Results from Other Laboratories

Other investigators reported that TSG improved impaired performance in the passive avoidance task, and inhibited the overexpression of APP in the hippocampus of AD-like model rats that were exposed to aluminum trichloride (AlCl3) in their drinking water [54]. TSG also rescued the learning and memory deficits caused by intracerebroventricular injection of Aβ in rats and might be connected to the synaptic changes in the hippocampus [55]. TSG ameliorated learning and memory of aged rats and might be related to the APP pathway [56]. Preincubation of BV-2 cells with TSG attenuated lipopolysaccharide (LPS)-mediated induction of TNF-α and IL-6 in microglia by reducing the binding activity of nuclear factor kappa B (NF-κB), and decreased the expression of inducible NOS (iNOS) and the level of NO [57].

3.8. Summary

These results indicate that TSG treatment improves learning and memory impairment in several AD-like animal models. TSG acts on multiple targets involved in the complicated pathogenesis of AD; it prevents or delays neuronal degeneration and death by enhancing cholinergic function, inhibiting the Aβ cascade and tau hyperphosphorylation, acting as an anti-inflammatory, anti-oxidative, and neurotrophic agent, and protecting synapses and mitochondria. TSG also ameliorates movement disorder in a PD animal model and inhibits α-synuclein overexpression and aggregation, suggesting that TSG may potentially be used to treat PDD and DLB, in which using an AChE inhibitor is not suitable. Completed phase II clinical trial of TSG in China demonstrated that TSG treatment effectively improved cognitive impairment in patients with mild to moderate AD, and the phase III clinical trial has now begun.

4. EPIMEDIUM EXTRACT

In TCM, *Epimedium* (Chinese name: Yinyanghuo) is used to tonify the kidney, invigorate *yang*, and strengthen muscles and bones. Epimedium flavonoids (EF) are the major active ingredient extracted from the *Epimedium* leaves and stems, and icariin (ICA) is the main component of EF.

4.1. Effects on Aβ Cascade

To investigate the effects of EF and ICA on the Aβ cascade, we used APPV717I transgenic mice. We found that oral administration of EF and ICA improved learning and memory, reduced the number of amyloid plaques, Aβ content, and β-secretase expression, inhibited α-synuclein overexpression and aggregation, protected synaptic structure, and increased the expression of synaptophysin and PSD95 in the hippocampus and cortex of transgenic model mice [58-60]. EF treatment also inhibited β-secretase activity *in vitro*, and decreased Aβ formation and secretion in APP695-transfected SH-SY5Y nerve cells [61]. In an Aβ1-40 intracerebroventricular injection mouse model, we discovered that EF improved learning and memory, inhibited the activation of microglia and astrocytes, decreased the IL-1β and TNF-α content, inhibited COX-2 expression, and increased GSH content and SOD activity in the hippocampus and cerebral cortex of the model mice [62, 63].

4.2. Effects on Mitochondrial Deficiency-Induced AD Model

To explore the effects of ICA on mitochondrial dysfunction, we used rats that were subcutaneously infused with sodium azide, an inhibitor of mitochondrial cytochrome c oxidase (complex IV). We found that ICA increased mitochondrial cytochrome c oxidase activity, decreased Aβ content and the expression of β-secretase and PS1, and enhanced the expression of NGF, BDNF, and TrkB in the hippocampus and cerebral cortex of model rats [38, 64].

4.3. Effects on Inflammatory Demyelination Model

In a rat model of experimental autoimmune encephalomyelitis induced by myelin basic protein (MBP), we discovered that EF treatment reduced the score of neurological deficits, alleviated demyelination and inflammatory infiltration, and inhibited the activation of astrocytes and the levels of IL-1β, TNF-α, NF-κB, and NO, enhanced the expression of NGF, increased the number of oligodendrocytes, and protected the ultrastructure of myelin sheaths and axons [65, 66]. These results suggest that EF may reduce neuroinflammation and enhance myelination in the central nervous system (CNS).

4.4. Effects on α-Synuclein and Neural Stem Cells *In vitro*

Our *in vitro* studies showed that ICA incubation with α-synuclein-transfected PC12 cells reduced α -synuclein mRNA and protein expression, inhibited α-synuclein aggregation, and increased the expression of Parkin and UCH-L1 in the ubiquitin-proteasome system [67]. In primary cultures of neural stem cells isolated from the hippocampus of neonatal rats, EF promoted the proliferation and differentiation of neural stem cells [68].

4.5. Results from Other Laboratories

Other investigators reported that oral administration of EF improved learning and memory function, increased Bcl-2 expression, and decreased Bax expression in the hippocampus of an AD rat model induced by Aβ1-40 intracerebroventricular injection [69]. ICA treatment ameliorated memory impairment in AD model mice (5xFAD) [70], and reduced Aβ content and β-secretase mRNA level in the hippocampus of an AD rat model induced by Aβ25-35 injection into the hippocampus [71]. ICA also suppressed abnormal inward calcium currents induced by Aβ25-35 in neonatal rat hippocampal slice CA1 pyramidal neurons [72]. *In vitro*, ICA treatment restored Aβ1-42-induced atrophy of axons and dendrites in rat cortical neurons [70].

Oral administration of ICA improved learning and memory, and increased the levels of acetylcholine (ACh) and ChAT in the cerebral cortex of SAMP10 mice, a senescence-accelerated mouse model [73]. ICA treatment decreased AChE content and activity, and prevented the decline of ChAT expression in the hippocampus of rats exposed to AlCl3 in their drinking water [74]. ICA suppressed the activation of astrocytes, and decreased the levels of TNF-α and IL-6 in the hippocampus of rats subjected to lipopolysaccharide (LPS) injection into the lateral cerebral ventricle [75].

4.6. Summary

These results demonstrate that EF and ICA ameliorates learning and memory dysfunction in AD-like animal models. Their mechanisms of action are involved in enhancing cholinergic function, inhibiting the Aβ cascade and α-synuclein aggregation, reducing inflammation and oxidative stress, protecting mitochondria and synapses, alleviating demyelination and white matter lesions, increasing neurotrophic factors, and promoting the proliferation and differentiation of neural stem cells.

5. CORNUS OFFICINALIS EXTRACT

In TCM, *Cornus officinalis* (Chinese name: Shanzhuyu) is used to tonify the liver and the kidney, and to arrest spontaneous emission and sweating. Cornel iridoid glycoside

(CIG), which mainly contains morroniside and loganin, is the major active ingredient extracted from the sarcocarp of *Cornus officinali*. We recently investigated the effects of CIG on several AD-like animal and cellular models.

5.1. Effects on Cholinergic Damage-Induced AD Model

Fimbria–fornix transection (FFT) deprives the hippocampus of its major cholinergic input and disrupts substantial components of hippocampal output, and thus establishes a classic AD model. In our experiments using FFT rats, oral administration of CIG improved learning and memory capacity, attenuated neuron loss, increased the expression of synaptophysin, NGF and its receptor TrkA, BDNF and its receptor TrkB, and growth-associated protein 43 (GAP-43), reduced the axon growth inhibitory factors Nogo A and chondroitin sulfate proteoglycan (CSPG), enhanced Bcl-2 and inhibited Bax and cytochrome c expression in the hippocampus of model rats [76-78].

5.2. Effects on Tau Hyperphosphorylation

Tau hyperphosphorylation plays an important role in the pathogenesis of AD [79], and is regulated by the activities of protein kinases and phosphatases. In an AD-like cell model induced by okadaic acid (OA), a PP2A inhibitor, we found that preincubation of human neuroblastoma SK-N-SH cells with CIG inhibited hyperphosphorylation of tau protein and neurofilament, and protected the neuronal cytoskeletal structure [80]. CIG also inhibited apoptosis by increasing Bcl-2 expression, and decreasing Bax and caspase-3 expression in OA-treated cells [81]. In SK-N-SH cells co-treated with wortmannin, a phosphatidylinositol-3 kinase (PI3K) inhibitor, and GF-109203X, a protein kinase C (PKC) inhibitor, CIG attenuated tau hyperphosphorylation at Thr205, Thr212, Ser214, Thr217, Ser396 and paired helical filament-1 (PHF-1), and improved morphological and microtubular cytoskeleton damage in SK-N-SH nerve cells; its mechanisms were involved in elevating the activity of PP2A by reducing the demethylation of PP2A catalytic subunit (PP2Ac) and the ratio of protein phosphatase methylesterase-1 (PME-1)/leucine carboxyl methyltransferase (LCMT) [82].

5.3. Effects on Cerebral Ischemia-Induced Dementia Model

We employed a chronic cerebral ischemia gerbil model, which was generated by bilateral common carotid artery occlusion and reperfusion. We found that CIG ameliorated learning and memory impairment, increased the number of neurons, decreased neuronal apoptosis via elevating Bcl-2 and reducing Bax and caspase-3 expression, enhanced the expression of BDNF, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), PI3K and Akt in the neuronal survival signal transduction pathway in the hippocampus of ischemic model gerbils [83, 84].

5.4. Effects on Neurogenesis

We discovered that CIG promoted neurogenesis and angiogenesis in chronic cerebral ischemic rats: CIG enhanced the proliferation and differentiation of endogenous neural stem cells (NSCs) in the subventricular zone (SVZ), cerebral cortex and striatum, increased the degree of neovascularization around the infarct, and enhanced the expression of VEGF and its receptor Flt-1, and BDNF and its receptor TrkB in the cerebral cortex of rats 28 days after cerebral ischemia [85-87]. In addition, CIG also promoted the proliferation and differentiation of endogenous NSCs in hippocampal dentate gyrus of normal adult rats [88].

5.5. Effects on Brain Injury Model

In the acute brain injury studies, we used a cerebral ischemia rat model and a traumatic brain injury rat model. CIG improved the neurological function, increased the number of surviving neurons, reduced inflammation by decreasing the activation of microglia and astrocytes as well as the levels of IL-1β and TNF-α, and suppressed neuronal apoptosis by increasing Bcl-2 expression and inhibiting Bax, caspase-3 and cytochrome c expression either in the cerebral ischemia model rats [89, 90] or in the traumatic brain injury model rats [91].

5.6. Effects on Inflammatory Demyelination Model

To investigate anti-inflammatory effects of CIG, we used an experimental autoimmune encephalomyelitis rat model induced by MBP. We found that CIG treatment reduced microglial activation and the levels of TNF-α, IL-1β and NF-κB, inhibited the JAK/STAT signaling system, and increased the expression of BDNF and the number of mature oligodendrocytes in the brain and spinal cord of model rats [92].

5.7. Summary

These results demonstrate that CIG improves cognitive impairment in AD-like animal models, inhibits tau hyperphosphorylation, reduces inflammation and apoptosis, increases neurotrophic factors, improves the microenvironment of central nerve regeneration, and promotes neurogenesis.

6. GINSENG EXTRACTS

In TCM, *Ginseng* (Chinese name: Renshen), a well-known Chinese medicinal herb, is used to reinforce *qi*, restore from collapse, tonify the lung and spleen, and to relieve mental stress. Ginsenosides, which contain about 30 monomers (e.g., Rbl, Rg1, Rg5, Rh2, Rd), are the main active ingredient extracted from *Ginseng* roots.

6.1. Effects on Aβ Cascade

In studies that targeted the Aβ cascade, intraperitoneal injection of ginsenoside Rh2 reduced senile plaques in the hippocampus of APP transgenic (Tg2576) mice. *In vitro* experiments showed that Rh2 treatment increased the soluble APPα (sAPPα) level and the C-terminal membrane-bound fragment (CTF) α/β ratio, and reduced Aβ concentration [93]. In rats that received bilateral injection of Aβ1-42 into the CA1 region of the hippocampus, the intraperitoneal administration of ginsenoside Rg1 decreased the Aβ1-42 level by up-regulating the expression of peroxisome proliferator-activated receptor γ (PPARγ) and insulin-degrading enzyme (IDE) in the hippocampus [94]. Rg1 treatment decreased the levels of Aβ1-40 and Aβ1-42 [95] secreted in N2a-APP695 cells *in vitro*, and reduced the expression of β -site APP-cleaving enzyme 1

(BACE1, β-secretase) mRNA and protein as well as β-CTFs (a C-terminal APP fragment cleaved by BACE1) in the model cells [96]. Rbl treatment ameliorated learning and memory impairment, and prevented the decline in ChAT activity in the cortex and hippocampus of mice that received an intracerebroventricular injection of Aβ25-35 [97].

6.2. Effects on Tau Hyperphosphorylation

In experiments that targeted tau hyperphosphorylation, Rg1 treatment improved memory impairment in rats subjected to intracerebroventricular or hippocampal injection of OA, and decreased tau hyperphosphorylation via inhibiting the GSK-3β signaling pathway or by increasing PP2A activity in the hippocampus [98, 99]. Preincubation of rat brain slices with Rg1 decreased tau hyperphosphorylation and caspase-3 expression induced by OA [100]. Oral administration of Rg1 decreased the production of neurofibrillary tangles (NFTs) and the phosphorylation of tau in retinal pigment epithelial cells by increasing neprilysin activity and decreasing PKA activity in an APP/PS1 double transgenic mouse model [101]. In cultured rat cortical neurons, Rb1 pretreatment decreased tau phosphorylation induced by Aβ25-35 treatment via inhibiting p25/cdk5 or through the JNK/p38 mitogen-activated protein kinase (MAPK) pathway [102, 103]. Rd inhibited tau phosphorylation in Aβ-treated cultured cortical neurons by altering the functional balance of GSK-3β and PP2A [104]. Rd treatment ameliorated ischemia-induced behavior impairment, and attenuated tau hyperphosphorylation by inhibiting the activity of GSK-3β and enhancing the activity of protein kinase B (PKB/Akt), a key kinase suppressing GSK-3β activity, in the brain of rats with focal cerebral ischemia [105].

6.3. Effects on Streptozotocin-Induced AD Model

Treatment with Rg5 improved cognitive dysfunction in rats that received an intracerebroventricular injection of streptozotocin, decreased Aβ deposition and the levels of TNF-α and IL-1β, and reduced AChE activity in the cortex and hippocampus of model rats [106].

6.4. Results from Our Group

We also found that ginsenosides inhibited the hyperphosphorylation of tau protein and protected the cytoskeleton in OA-treated human neuroblastoma SK-N-SH cells [107]. Rg1 treatment attenuated tau phosphorylation and apoptosis in SK-N-SH cells treated with the supernatant collected from Aβ1-40-stimulated THP-1 monocytes, and decreased Aβ-induced inflammation and the content of IL-1β and TNF-α in THP-1 cells [108-110].

6.5. Summary

These results indicate that ginsenoside ameliorates learning and memory impairment in AD-like animal models, inhibits the Aβ cascade and tau hyperphosphorylation, reduces inflammation, and enhances cholinergic function.

7. PUERARIA EXTRACT

In TCM, *Pueraria* (Chinese name: Gegen) is used to eliminate pathogenic factors from the superficial muscles and reduce heat, as well as to promote salivation and relieve thirst. Puerarin is the major active component extracted from *Pueraria* root, and is used clinically in China to treat ischemic cardiovascular disease and cerebrovascular disease.

7.1. Effects on Aβ Cascade

In studies that targeted the Aβ cascade, oral administration of puerarin ameliorated cognitive impairment via activation of the Akt/GSK-3β signaling pathway in APP/PS1 transgenic mice. Further, puerarin treatment decreased the level of lipid peroxidation by inducing the nuclear factor erythroid 2-related factor 2 (Nrf2) target gene heme oxygenase 1 (HO-1) in the hippocampus [111]. Puerarin treatment improved Aβ-induced cognitive impairment and reversed the increase in neuronal apoptosis and caspase-9 activation in the hippocampus of rats that received intrahippocampal Aβ injections [112, 113] and in mice subjected to Aβ intracerebroventricular injection [114].

In vitro experiments showed that puerarin pretreatment inhibited Aβ25-35-induced neurotoxicity and apoptosis in PC12 cells, enhanced the expression of p-Akt and p-Bad, increased the Bcl-2/Bax ratio, and reduced caspase-3 activation and cytochrome c content [115, 116]. Puerarin protected against Aβ-induced microglia apoptosis via a PI3K-dependent signaling pathway [117].

7.2. Effects on Oxidative Stress

Puerarin ameliorated learning and memory deficits in senescence-accelerated mice (SAMP-8) [118] and in an AlCl3-injected mouse model, as well as increased SOD activity and decreased malondialdehyde (MDA) [119] in the hippocampus [120]. In rat primary hippocampal neurons, puerarin pretreatment reduced Aβ25-35-induced oxidative stress by scavenging ROS and inhibiting lipid peroxidation, induced expression of the nuclear Nrf2 protein, increased the HO-1 level, and stimulated Serine 9 phosphorylation of GSK-3β [121]. The preventive property of puerarin against oxidative stress-induced neurodegeneration was verified using mitochondrial transgenic neuronal cybrids of sporadic AD [122]. Puerarin together with ligustrazine decreased the MDA level and LDH leakage, and increased SOD activity in primary hippocampal neurons exposed to Aβ25-35 [123].

7.3. Summary

These results demonstrate that puerarin also improves learning and memory impairment in AD-like animal models, inhibits the Aβ cascade, and reduces oxidative stress and apoptosis, in addition to treating ischemic cardio-/ cerebrovascular diseases.

8. CLAUSENA LANSIUM EXTRACT

Clausena lansium (Chinese name: Huangpi) is a fruit that grows in the southern part of China. In TCM, its root, leaf, and seeds promote *qi* circulation and relieve pain, dissipate heat, and eliminate phlegm. Chemists have isolated several compounds from the aqueous leaf extract, including (−) clausenamide. The partial chemical structure of this compound is similar to the pharmacophore of piracetam, a

nootropic drug developed in Europe. The compound was chemically synthesized in China [124].

8.1. Effects on Cholinergic Function

Pretreatment with (−)clausenamide improved memory deficits in mice that received an intraperitoneal injection of anisodine, an anticholinergic drug. Further, (−)clausenamide ameliorated ACh reduction in the brain; (+)clausenamide had no effect on ACh decrease [125]. *In vitro* study showed that (−)clausenamide increased ChAT activity and content in cultured rat frontal cortex neurons, stimulated proliferation of neuronal cells, and supported the survival and neurite outgrowth of neurons [126].

8.2. Effects on Synaptic Plasticity

In the electrophysiological study, (−)clausenamide potentiated basic synaptic transmission and high frequency stimulation-induced LTP [127], increased hippocampal synapses and Mossy fiber spouting in rats, but (+)clausenamide had no neurotrophic action [128]. Intracerebroventricular administration of (−)-7-OH-clausenamide increased population spike (PS) amplitude in extracellular recordings from the hippocampal dentate gyrus following application of low-frequency stimulation; however, (+)-7-OH-clausenamide decreased PS amplitude; basal transmission in the hippocampus could be potentiated by (−)-7-OH-clausenamide and attenuated by (+)-7-OH-clausenamide. These findings indicated that there was a stereoselective difference between the two enantiomers in the modulation of synaptic responses and plasticity [129].

8.3. Effects on Aβ Cascade

In experiments that examined the Aβ cascade, oral administration of clausenamide improved the learning and memory dysfunction in an Aβ25-35-exposed rat model [130] as well as in a diabetes model where rats were administered an intraperitoneal injection of streptozotocin [131]. (−)Clausenamide treatment enhanced cognitive function and protected neurons in the hippocampus and cerebral cortex in rats that were subjected to ovariectomy and intracerebroventriclar injection of Aβ25-35 [132]. Aβ25-35 induced the apoptotic cascade in differentiated PC12 cells, and (−)clausenamide incubation reversed calcium overload, prevented ROS generation, moderated the dissipation of mitochondrial transmembrane potential and the imbalance of Bcl-2 and Bax, inhibited the activation of p38 MAPK and p53 expression, and cleaved caspase-3 [133].

8.4. Effects on Tau Hyperphosphorylation

(−)Clausenamide pretreatment inhibited tau hyperphosphorylation at serl99/202 and ser396, and decreased the cell death rate and apoptosis in OA-treated human neuroblastoma SH-SY5Y cells [132]. (−)Clausenamide incubation increased PC12 cell survival rate and inhibited apoptosis induced by serum deprivation via elevating Bcl-2 expression and decreasing the expression of Bax and GSK-3β [134].

8.5. Summary

These results demonstrate that (−)clausenamide treatment improves learning and memory in AD-like animal models, increases cholinergic function, enhances synaptic plasticity, and inhibits the Aβ cascade, tau hyperphosphorylation, and apoptosis. The phase I clinical trial of (−)clausenamide is complete in China.

9. HUPERZIA SERRATA EXTRACT

In TCM, *Huperzia serrata* (Chinese name: Qiancengta) is used to eliminate blood stasis and stop bleeding, relieve swelling and pain, and clear away heat and toxic material. Huperzine A (Hup A) is an alkaloid extracted from *Huperzia serrata*. Multiple lines of evidence demonstrate that Hup A is a competitive and reversible AChE inhibitor. Hup A is used clinically in China to improve memory impairment in aged people and in patients with mild to moderate AD. The novel action mechanisms of Hup A have been explored recently.

9.1. Effects on Oxidative Stress

In vitro studies using neuron-like rat pheochromocytoma PC12 cells and cultured rat primary cortical neurons demonstrated that Hup A attenuated Aβ-induced oxidative injury, enhanced cell viability and the activities of antioxidant enzymes, including SOD, GSH peroxidase and catalase, as well as decreased the level of MDA [135, 136]. Oral administration of Hup A improved learning and memory, increased GDNF mRNA expression and SOD activity, and decreased MDA and NO content in the brain tissue of aged rats [137] and vascular dementia model rats [138, 139].

9.2. Effects on Aβ Cascade

A recent study verified the beneficial effects of Hup A on mitochondrial dysfunction and memory deficits in APP/PS1 double transgenic mice at a time point in which AChE was not inhibited [140]. Hup A treatment reduced amyloid plaque burden, Aβ level and hyperphosphorylated tau expression, and alleviated synaptic deficits in the cortex and hippocampus of APP/PS1 transgenic mice [141]. Hup A treatment also decreased iron content in the brain of APP/PS1 transgenic mice, and reduced the expression of transferrin-receptor 1, as well as transferrin-bound iron uptake in cultured neurons exposed to iron. Therefore, reducing iron in the brain may be a novel pharmacologic mechanism of Hup A for the treatment of AD [142].

9.3. Summary

These results indicate that Hup A also acts as an antioxidant, protects mitochondria, inhibits the Aβ cascade and reduces iron, in addition to inhibiting AChE.

10. ANEMARRHENAE EXTRACT

In TCM, *Anemarrhenae* (Chinese name: Zhimu) is used to nourish *yin* and lessen fire, and for moistening and laxation. Numerous steroids have been extracted from the *Anemarrhenae* rhizome, including sarsasapogenin, markosapogenin, and negitogenin. Their glycosylated products include timosaponin-AI, -AII, -AIII, -AIV, -BI, and -BII.

10.1. Effects on Cholinergic Damage-Induced AD Model

To establish dementia model, a combination of Aβ25-35 and ibotenic acid was injected into the right nucleus basalis

in rats. Oral administration of sarsasapogenin improved learning and memory, and increased ChAT activity and muscarinic receptor density in the cerebral cortex, hippocampus, and striatum of the model rats [143, 144]. Incubation of sarsasapogenin up-regulated muscarinic M1 receptor density in aged CHO cells by promoting CREB production and phosphorylation [145]. Timosaponin-AIII treatment reversed the learning and memory deficits induced by scopolamine in mice, increased ACh level, inhibited AChE activity, and decreased TNF-α and IL-1β content in the brain [146]. *In vitro* study showed that timosaponin-AIII inhibited the activation of NF-κB signaling induced by TNF-α in BV-2 microglia and by scopolamine in SK-N-SH neuroblastoma cells [146].

10.2. Effects on Aβ Cascade

Saponins from *Anemarrhena asphodeloides* Bunge (SAaB) improved spatial learning and memory impairment induced by Aβ1-42 in aged rats, and inhibited the activation of astrocytes in the hippocampal CA1 region [147]. SAaB incubation suppressed apoptosis and the expression of phospho-ERK1/2 and phospho-p38 MAPK proteins, and decreased the levels of TNF-α, NO, and iNOS in cultured mouse peritoneal macrophages stimulated by Aβ25-25 [148]. SAaB ameliorated memory deficits and decreased the expression of β-APP in the hippocampus of rats subjected to AlCl3 [149].

Timosaponin-B treatment decreased phosphorylated tau-positive cells following Aβ25-35 injection into the hippocampus of rats [150]. Timosaponin-BII treatment improved neuronal metabolic activity, decreased AChE activity, LDH leakage and MDA production, and increased SOD activity in primary neurons damaged by Aβ25-35 [151]. Intravenous injection of timosaponin-BII inhibited the up-regulation of BACE1, decreased the accumulation of Aβ1-40 and β-CTF, and decreased the level of MDA induced by ferric chloride (FeCl3) in rat retina [152].

10.3. Effects on Inflammation

Oral administration of SAaB improved learning and memory, attenuated pyramidal neuron damage, and reversed the increases in IL-1β and iNOS activation in hippocampal CA1 region induced by LPS intracerebroventricular injection in rats [153].

10.4. Effects on Aging Model

In the experiments on aged rats, SAaB treatment enhanced the learning and memory ability, elevated nicotinic cholinergic receptors and BDNF content in the brain [154, 155]; increased synaptophysin and PSD95 expression, and up-regulated Akt/mammalian target of rapamycin (mTOR) signaling pathway in hippocampal CA3 region of aged rats [156].

10.5. Summary

These results demonstrate that timosaponin improves learning and memory impairments in AD-like animal models, enhances cholinergic function, inhibits the Aβ cascade, and reduces oxidative stress as well as inflammation.

CONCLUSION

AD is a multifactorial complex disease. TCM studies performed by our group and in other laboratories demonstrated that a new compound drug (Shenwu capsule) and approximately 10 Chinese medicinal herb extracts (tetrahydroxystilbene glucoside, epimedium flavonoid, icariin, cornel iridoid glycoside, ginsenoside, puerarin, clausenamide, huperzine A, and timosaponin) have beneficial effects on multiple targets involved in the complicated pathogenesis of AD. These compounds prevent or delay neuronal degeneration and death, and improve learning and memory impairment in AD-like animal models by enhancing cholinergic function, inhibiting the Aβ cascade and tau hyperphosphorylation, and protecting synapses and mitochondria. These compounds also exert anti-inflammatory, anti-oxidative, and neurotrophic effects.

These results suggest that TCM may play an important role in AD therapy and have broad application prospects. Firstly, compound drugs for AD treatment can be composed of several traditional Chinese drugs based on TCM theory in combination with modern pharmacological researches. Secondly, new drugs for AD therapy can be developed by combining several TCM herb ingredients or components acting on different targets in AD pathogenesis. Thirdly, a multi-target ingredient or component from single TCM herb can also be developed as a new drug for AD therapy. Nevertheless, more researches on chemical compositions, pharmacological actions, molecular mechanisms, safety and standard clinical trials of TCM drugs are still needed.

In addition, our experiments demonstrated that the extracts (TSG, EF, ICA, and CIG) from some "kidney-tonifying" TCM drugs (*Polygonum multiflorum, Epimedium,* and *Cornus officinalis*) have both neuro-protective and neurotrophic/neuro-regenerative effects. This may partially provide the modern biological bases for TCM theory that "the kidney nourishes marrow and the brain is the sea of marrow" [157]. These discoveries also suggest that some "kidney-tonifying" TCM drugs and their extracts may be beneficial for treatment of neurodegenerative diseases and nerve injury.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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