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•Reviews•

Research advances in the treatment of Alzheimer's disease with polysaccharides from traditional Chinese medicine

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[ABSTRACT] Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the loss of patients' memory and their cognitive abilities and the mechanism is not completely clear. Although a variety of drugs have been approved for the AD treatment, substances which can prevent and cure AD are still in great need. The effect of polysaccharides from traditional Chinese medicine (TCM) on anti-AD has gained great progress and attained more and more attention in recent years. In this review, research advances in TCM-polysaccharides on AD made in this decade are summarized.

[KEY WORDS] Alzheimer's disease; Polysaccharides; Traditional Chinese medicine
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Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease that gradually destroys brain cells or connections between brain cells, leading to progressive loss of patients' memory and cognitive functions, even the change of their personalities. The average duration of AD is approximately 7−10 years, including mild, moderate, and severe stages. It is the most common form of dementia and accounts for about 70% of dementia cases, especially in people aged 65 and over. But AD is not just a normal part of aging. There are over 35 million AD patients worldwide and the number is expected to grow dramatically as the population ages [1]. AD is characterized by the extracellular senile plaques (SP) and intracellular neurofibrillary tangles (NFT) [2]. Plaques are composed of the aggregates of amyloid β-peptide (Aβ) and deposits of fibrils, while NFTs result from the hyperphosphorylated microtubule-associated protein tau [3]. The disease mechanism is complicated and still unclear. There are several competing hypotheses, including genetics, amyloid hypothesis, cholinergic hypothesis, free radical damage hypothesis, calcium homeostasis imbalance hypothesis, and apoptosis hypothesis [4-5]. Medications currently used to treat AD include cholinesterase inhibitors, *N*-methyl-D-aspartic acid receptor (NMDA), Aβ aggregation inhibitors, antioxidant, therapeutics intervening abnormal phosphorylation of tau, and neurotrophic factors [6-7]. Despite great progress over the past decades, there are still no effective treatments to prevent, halt, and reverse AD.

Traditional Chinese medicine (TCM) is a treasure in China, with abundant resources. Decoction is the most common and important formula in TCM, and polysaccharide is one of the main components of soluble substance in decoction. Carbohydrates are getting more and more attention in 21st century. It has been demonstrated that carbohydrates not only act as energy resource, but also play vital roles in the protein folding, secretion, recognition of biological macromolecules and interactions [8]. Therefore, they affect cell growth and, differentiation, morphogenesis, migration, and signal transduction, etc [9]. So far natural carbohydrates have been reported to have multiple bioactivities such as anti-tumor,

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anti-virus, anti-dementia, anti-inflammatory, and anticoagulation, without obvious side effects.

One of the most significant characteristics of TCM is that TCM are usually composed of multiple active ingredients. They function to treat disease by multiple pathways and on multiple targets. Thus, it is reasonable to use TCM to address AD that has complicated mechanism and a variety of pathogenesis, since it has the advantage over treatment focusing on single target. This review will summarize the bioactivities and mechanism studies of TCM-polysaccharide in the treatment of AD in the decades, discuss challenges at present and prospect in the near future, and provide the foundation information for the research and development of carbohydrate-based drug of AD.

TCM-polysaccharides treatment

Targeting amyloid β-peptide (Aβ)

Aβ peptide, the main constituent of senile plaques, denotes peptides of 36−43 amino acids that are crucially involved in AD and derived from amyloid precursor protein (APP) by the proteolytic cleavage of β- and γ-secretase. Aβ exists in the cerebrospinal fluid at low concentration and provides nutrition for immature neurons. Because of the imbalance of its synthesis, catabolism, and transportation, Aβ aggregates and deposits in brain tissue, which are hallmark of the disease, triggering a cascade and leading to the dysfunction and death of neurons. It has been demonstrated that Aβ might be the most toxic in the form of soluble oligmers in the early stage of aggregation. The gene mutation of APP, presenilin-1 (PS-1) and preseniline-2 (PS-2), especially that of PS-1, results in an increase in Aβ, which is proven to be the main reason for early-onset AD. Therefore, it has been a hot spot to delay and alleviate AD to reduce Aβ formation and deposition and to inhibit its aggregation [10-12].

Interestingly, Aβ has been notorious for its toxicity for decades, but Kumar *et al.* have recently shown that Aβ is a natural antibiotic protecting the brain from infection and Aβ aggregates trap bacterial pathogens. These new findings identify inflammatory pathways as potential new drug targets for treating AD [13].

Drugs targeting Aβ are predominant at present. 31 molecules targeting Aβ metabolism or Aβ itself are across the clinical developmental stages, including 9 therapeutics targeting APP metabolism (such as α-secretase activators, β-secretase inhibitors, γ-secretase inhibitors, and modulators) and other compounds inhibiting Aβ oligomerization and aggregation, or promoting Aβ clearance. 5 out of 7 drugs in phase III trials are related to Aβ aggregation or clearance [6].

A homogeneous polysaccharide LJW0F2 isolated from flowers of *Lonicera japonica* Thunb. was elucidated to be an alpha-D-(1→4)-glucan with an alpha-(1→4) linked branch attached to the C-6 position. It could inhibit Aβ_{42} aggregation in a dose-dependent manner and attenuate the cytotoxicity induced by Aβ_{42} aggregation in SH-SY5Y neuroblastoma cells [14]. Moreover, it could also inhibit Aβ_{42} secretion in CHO/APPBACE1 cells and the expression of APP and BACE1. Polysaccharide from Vitis viniferal (VTP) and *Qing Xin Kai Qiao Fang* exert a protective effect on neurons against the injury induced by Aβ_{25-35} by adjusting APP mRNA expression and inhibiting Aβ formation [15-17]. Xiao *et al*. have found Kadsura heteroclite polysaccharide significantly inhibitsd Aβ_{42} production in M146L cell dose-dependently [18]. Huang has studied polysaccharide extracted from *Millettia Pulchra Kurz* var. Laxior (Dunn) Z Wei, an ethnic drug in Guangxi province, LYS polysaccharide (LYSP) [19]. The results show that LYSP inhibits the expression of APP, PS1 and PS2 dose-dependently, so as to reduce the over-production of Aβ in the brain and protect the neurons from the neurotoxicity of Aβ in senescence accelerated-prone mouse/8(SAMP8). Polygonapolysaccharose, ganoderma lucidum polysaccharide peptide (GLPP), and astragalus polysaccharides (APS) could reduce the deposition of Aβ in the hippocampus [20-22]. A series of homogeneous short chain beta-(1, 4)-D-mannosyl oligosaccharides, derived from the marine plant oligomannurarate 971, show neuroprotective effect against Aβ peptide toxicity in SH-SY5Y human neuroblastoma cells [23].

Targeting Tau proteins

Tau proteins are highly soluble microtubule-associated protein (MAP) and are abundant in neurons [24]. They interact with tubulin to stabilize microtubules and promote tubulin assembly into microtubules. In humans, the gene encoding tau protein is located on chromosome17, containing 16 exons [25]. Tau protein is phosphorylated and there are 2−3 phosphorylation sites in each microtubule. However, hyperphosphorylation can result in the self-assembly of tangles of paired helical filaments (PHF) and further neurofibrillary tangles (NFT), which are the pathological characteristics of AD [26]. Dermaut has proposed that hyperphosphorylation of tau and the amount of NFT are positively associated with AD dementia degree [27]. It is thus one of hotspots for AD treatment to prevent tau hyperphosphorylation. Recently Brier *et al*. have found that Tau deposition in the temporal lobe is more closely tracked dementia status, as a better predictor of cognitive performance than Aβ deposition in any region of the brain [28].

The degree of tau phosphorylation is in dynamic equilibrium between phosphorylation by protein kinases and dephosphorylation by protein phosphatase [29]. In consequence, therapeutics targeting tau for AD treatment include proteinase inhibitor, improvement of protein phosphorylase activity, inhibition of tau aggregation into NFTs and increasing tau degradation [30]. Tau-related therapeutics has been growing steadily in recent years although fewer drug trials have focused on tau. Recently Rmeber, the inhibitor of tau protein aggregation and NAP, microtubule stabilizer, are in clinical Phase III and II trials, respectively [31].

Interestingly polysaccharide from *Cornus officinalis* might significantly impedes the hyperphosphorylation of tau protein at Ser422 and Ser396 epitope, and reduces the expres-

sion of glycogen synthase kinase-3β (GSK-3β) in hippocampus of AD mice [32-33]. Chen *et al*. have found that polysaccharides from Yulangsan (YLSP) reduces the number of pSer202 positive cells, i.e. the abnormal Ser202 phosphorylation and ameliorates the neuronal loss in the frontal lobe and hippocampus of SAMP8 [34]. Besides, polysaccharide peptide from *Ganoderma lucidum*, GLPP, has been reported to inhibit p-tau Ser396/Ser404 and p-tau Ser199/Ser202 expression [21].

Targeting cholinergic neurotransmitter

Cholinergic neurotransmitter is an important substance in brain involved with the cardiovascular activities, regulation of motility and sensation, the physiological process of learning and memory, etc [35]. It has been extensively documented that AD typically has deterioration and a decline in the cholinergic neuron, especially in basal forebrain areas, hippocampus and neocortex [36]. Acetyl choline (Ach) is one of the most important neurotransmitters in the brain and has close relation to the cognition [37]. Acetylcholine esterase (AchE) and choline acetytransferase (ChAT) are important enzymes catalyzing hydrolysis and synthesis of Ach, respectively. The abnormal expression of AchE and ChAT leads to the disorder of Ach synthesis and release, with the symptoms of declining recognition function and memory disorder [38].

A variety of treatments have been applied to improve the activity of the cholinergic neurons, including stimulating Ach synthesis by choline and phosphatidylcholine supplements, inhibiting Ach degradation by AchE inhibitors, and protecting cholinergic neuron, etc [39]. AchE inhibitors are the most popular drugs which are currently used to treat the cognitive problems of AD, such as tacrine, donepezil, galantamine and rivastigmine [40].

Angelica sinensis polysaccharide inhibits the activity of AchE in the brain of senile dementia mice, so as to alleviate the learning and memory disorder [41]. Polysaccharide from Cistanche deserticola (CDPS) increases AchE content and ChAT activity in the cerebral cortex and hippocampus of AD mouse induced by Aβ_{1-40}, so as to improve significantly the learning ability and memory [42]. Zhao *et al*. have reported that Fudican polysaccharide sulfate (FPS) augments ChAT activity and inhibits AchE activity, thus increasing Ach content and improving the learning ability of AD mice [43].

Although most active gradient are reported to protect the nervous system by decreasing AchE activity and increasing Ach content, Liu has found that astragalus polysaccharides provide good intervention on learning and memory in vascular dementia mice *via* enhancing AchE activity and decreasing the Ach content in mice brain tissue [44].

Targeting oxidative stress

Oxidative stress (OS) was proposed for the first time in 1990 by Professor Sohal [45]. It refers to imbalance between oxidation and antioxydation, the overpowering of the antioxidative defense system by the oxidative system, which results in a large amount of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS interact with lipid, protein and nucleic acid, leading to cell dysfunction, tissue injury, and disease [46]. Common ROS are superoxide ($\cdot O_2^-$), hydroxyl radical ($\cdot$OH), hydrogen peroxide (H_2O_2) etc. and RNS includes nitric oxide ($\cdot$NO), nitrogen dioxide ($\cdot NO_2$) and peroxynitrite ($\cdot ONOO^-$), etc. Brain is one of organs consuming most oxygen and contains less antioxidase, which results in the weak free radical scavenging capacity and in the oxidation of unsaturated fatty acid in neuron [47]. Moreover, blood brain barrier (BBB) prevents the passage of some antioxidant from the bloodstream, so the brain is especially sensitive to oxidative stress, which is the main reason for a variety of neurodegenerative disease [48]. OS is obvious in each stage of AD and the oxidation degree increases with disease progression [49]. It has been reported that OS activates a positive feedback between the β- and γ-secretase cleavages of APP, thereby promoting production of Aβ [50]. In addition, ROS promotes hyperphosphorylation of tau by activating protein kinase excessively. Therefore, antioxidation might be an effective pathway for the development of therapies to treat or delay AD.

Antioxidants are usually classified into enzymatic and non-enzymatic systems [51]. The former includes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), etc. The latter includes vitamin, amino acid and metalloprotein, such as Vitamin E, renieratene, Vitamin C, systeine, tryptophan and lactoferrin, etc. Oxidation reaction chains are blocked to delay AD by such pathway as eliminating free radicals and/or decomposing hydroperoxide.

Yam polysaccharide increases significantly vitality of SOD, CAT Mg^{2+}-ATPase, Na^+-K^+-ATPase as well as the quotient of brain, and reduces the content of malondialdehyde (MDA) of AD mice induced by aluminum chloride [52]. The study on the antioxidant effect of YLSP on Aβ_{25-35} induced PC 12 cells injury has shown that in the cell culture medium and cell cytoplasm of YLSP-treated group, MDA and nitric oxide (NO) content as well as nitric oxide synthase (NOS) activity are decreased significantly, while SOD and GSH-Px activity as well as GSH content are increased greatly, so as to improve the cell viability [53]. Thus it is concluded that YLSP has obvious protective effects by scavenging free radicals [53-54]. Angelica polysaccharide enhances SOD and telomerase activity, thus improving the ability to scavenge free radicals and protecting cell from injury, so as to delay senescence and improve the study ability and memory [55]. LYSP enhances SOD and GSH-Px activities and decreases significantly MDA and NO contents in SAMP8 serum and brain, so as to scavenge effectively free radicals for anti-dementia goal [56]. Radix polygoni multiflori preparata polycose is reported to activate NOS in Ammon's horn, SOD and CAT activities, and reduce the level of cerebral lipofusin and monoamine oxidase, thus improving the learning ability and memory in a D-galactose induced senile dementia rabbit model [57-58]. Both *Ganoderma lucidum* polysaccharides and *Cistanche deserticola* polysaccharide (CDPS) are discovered to decrease MAD content and

increase SOD activity in the serum and brain of AD rats induced by A$\beta_{1\text{-}40}$ [59-61]. What's more, CDPS could significantly lower NO and ROS in the hippocampus region [59]. Tian *et al*. have studied effects of different fractions of Acori graminei rhizome extracts on learning and memory abilities of AD mice by injecting A$\beta_{1\text{-}42}$ into CA1 area of bilateral hippocampus. Their results show that both decoctum and essential oil fraction decrease NOS activity in the cerebrum and hippocampus, so as to improve learning and memory abilities. Polysaccharide is the one of the main components in water decoctum and its effects need to be confirmed further [62]. Dai *et al*. have proven Lentinan (LNT) improves the neuron cell livability after impairment with glutamate by antioxidation, and LNT significantly decreases LDH, NO and MDA content and increases SOD activity [63].

Polysaccharide is the main active ingredient in Optimized *Danggui Shaoyao San* (FBD), a Chinese herbal compound formula. It has notably protective effect on the H_2O_2-induced injury of PC12 and ECV304 cells in a dose-dependent manner and also raises the SOD activity and reduces MDA content in the brain of cyclophosphamide-induced injured mice, indicating that the mechanism of prevention of FBD from vascular dementia (VD) is based on the anti-oxidation of polysaccharides [64]. Xu *et al*. have studied the synergistic effect of polysaccharide from fleece flower root (PFR) and from Barbary wolfberry fruit (PBWF) on antisenility and its mechanism [65]. After treatment with mixture of PFR and PBWF in different ratios, activities of SOD and GSH-PX are ascended in kidneys and liver, while MDA and lipofuscin are descended in brain of senile model mice [65]. Therefore, the combination of PFR and PBWF exerts the synergistic effect on antisenility by eliminating oxygen free radical and active oxygen and by antilipoperoxidation.

Targeting anti-apoptosis

Apoptosis is the process of programmed cell death and plays an essential role in removing abnormal cells and maintaining the life activities. Apoptosis of neurons exerts a major role in neurodegenerative disorder like AD and leads to the loss of a large amount of neurons [66]. It is influenced by various factors such as caspases, Bax, Bcl2, Aβ, tumor necrosis factor-α (TNFα), reactive oxygen species, and perturbation of enzymes. Mechanisms of neuron apoptosis are involved the following: (1) abnormal cell cycle: neurons which has stopped differentiation reenter the cell cycle under pathological conditions such as oxidative stress, cerebral injury and overexpression of APP, thus leading to cell death via apoptosis; (2) disorder of gene expression; (3) other factors such as deposit of abnormal proteins and axonal and dendritic degradation [67].

Drugs under development with anti-apoptotic activity and those having potential application targeting apoptosis for AD treatment includes Huperzines, Lithium and GSK-3β inhibitors, anti-inflammatory drugs, leptin, bile acids and antioxidants as apoptotic inhibitors such as ginkgo biloba and melatonin, etc. [68]. Some emerging approaches also open up a new strategy for the future AD treatment such as neural stem cells transplantation and RNAi-induced gene silencing [69].

Yu *et al*. have demonstrated that homogeneous polysaccharides J2, J3, J4, and J6 purified from the flowers of *Nerium indicum* decrease cytotoxicity triggered by Aβ and activity of caspase-3, exerting neuroprotective effect against apoptosis in different pathways [70-71]. J2, J3, and J4 stimulate the phosphorylation of PDK-1 (Ser241) and Akt (Thr308) and may primarily rely on activation of Akt survival signaling pathway, while J6 inhibits Aβ-stimulated phosphorylation of c-Jun *N*-terminal kinase (JNK-1) and may rely on inactivation of JNK signaling pathway. YLSP has obviously protective effect on A$\beta_{25\text{-}35}$ induced apoptosis in PC12 cells, up-regulating anti-apoptotic gene Bcl-2 and down-regulating pro-apoptotic gene p53, Bax and caspase-3 [53, 72]. Moreover, Tau (Ser202) phosphorylation and caspase-3 expression and activity are reduced by YLSP in the frontal lobe and hippocampus of SAMP8, resulting in the reduction of neuron apoptosis [73]. *Angelica sinensis* polysaccharide, *Sargassum fusiforme* polysaccharide (SFPS) and *Vitis viniferal* polysaccharide (VTP) increase Bcl-2 and decrease Bax gene expression, so as to inhibit cell apoptosis [74-76]. *Ganodema lucidum* polysaccharide significantly down-regulates c-fos, caspase-3 and fasL gene expression, decreases apoptosis rate in hippocampal cells and increases the synaptophysin expression levels [77-80]. *Cistanche deserticola* polysaccharide (CDPS) upregulates Bcl-2 and down-regulates caspase-3 expression, so as to inhibit apoptosis of hippocampal neuron and improve learning ability of AD mice [81]. Polygona-polysaccharose could reduce neuron apoptosis in A$\beta_{1\text{-}42}$ induced AD rats' hippocampus and improve learning and memory ability [82]. Oligosaccharides of *Morinda Officinali* (OMO) increases the number of pyramidal neurons in hippocampus and the number of neuron cells in hippocampal CA1, cerebral cortex and basal nucleus of Meynert, which may result from the inhibition of the brain neuron apoptosis [83].

Erjingling (EJL), a Chinese herbal compound prescription, consists of the same amount of *Lycium barbarum* and polygonatum [84]. Polysaccharide extracted from EJL is the main active compound and raises the survival rate of hippocampal cells, up-regulates Bcl-2, down-regulates Bax and reduces the apoptosis of neurons, so as to protect hippocampal neurons from the apoptosis induced by glutamate [85]. Polysaccharide from formula *Qing Xin Kai Qiao Fang* decreases the expression of Bax and Caspase-3 in the cortex and hippocampus, so as to improve in learning and memory ability of AD rats [16].

Aqueous extract from *Lycium barbarum* LBA is reported to have a typical dose-dependent neuroprotective effects against toxicity of fibrillar A$\beta_{1\text{-}42}$ and A$\beta_{25\text{-}35}$ fragments by inhibiting the activation of c-Jun *N*-terminal kinase (JNK) [86]. The effective dosage of this extract is wider than that of a well-known western neuroprotective medicine lithium chlo-

ride (LiCl). However, the alkaline extract of *Lycium barbarum* LBB and two homogeneous polysaccharides LBB-I and LBB-II (sub-fractions purified from LBB) show the neuroprotective effects to attenuate Aβ peptide neurotoxicity by the stimulation of Akt signaling pathway instead of by inhibiting JNK signaling pathway as LBA [87]. Aqueous extract from *Verbena officinalis* Linn demonstrates neuroprotection effect against Aβ peptide toxicity and reduces both destruction of neuritis and neuronal apoptosis by attenuating Aβ activated PKR and JNK stress kinases [88].

Targeting calcium channel

Calcium ion is one of the most important signaling molecules and plays essential roles in life process such as cell proliferation, nerve transmissions, gene expression, and mitosis. The dysfunction of Ca^{2+} homeostasis activates a series of protein kinases and results in neuron damage, cell apoptosis and release of a large amount of excitatory neurotransmitter [89]. The calcium hypothesis of AD has been proven and developed. The study has demonstrated that the increase in calcium stimulates APP metabolism and AD mutations could induce changes in Ca^{2+} signaling in turn [90]. Moreover, enhancement of Ca^{2+} could activate protein kinase, leading to the imbalance between protein kinase and phosphatase and resulting in the tau hyperphosphorylation [91]. The alternation of Ca^{2+} signaling therefore contributes to the neurodegeneration and loss of learning and memory ability [92-93].

Owing to the fact that Ca^{2+} overload could induce a series of chain reaction to cause neuron apoptosis and lesion, calcium antagonist is a good way to inhibit Ca^{2+} increase to delay the neuron death and to treat AD. At present antagonists include nimodipine, verapamil, Flunarizine hydrochloride and tetrandrine, etc.

Ca^{2+} concentration is decreased while Ca^{2+}-ATPase and $Ca^{2+}Mg^{2+}$-ATPase increase in brain after 30-days intraperitoneal injection of Angelica polysaccharide (AP) for D-galactose and $NaNO_2$-induced senile dementia, which has demonstrated that AP down-regulates Ca^{2+} content by up-regulating Ca^{2+}-ATPase activity so as to get right the Ca^{2+} metabolism [41]. The concentration of intracellular calcium in PC12 treated with A$\beta_{25\text{-}35}$ is increased significantly, while that in YLSP-treated group is observed lower, demonstrating that YLSP could protect PC12 cells from calcium overload damage induced by A$\beta_{25\text{-}35}$ [94].

Other possible mechanisms

There are some other mechanisms reported besides above mentioned. Central neurotransmitter includes acetyl choline, monoamines, amino acids and peptides. Effects of LYSP on neurotransmitter in SAMP8 mice's brain are studied by continuous intragastric administration for 40 days [95]. Results show that LYSP up-regulates significantly monoamine neurotransmitter content of norepinephrine (NE), dopamine (DA) and 5-HT and down-regulates excitatory amino acids neurotransmitter content of glutamic acid (Glu) and aspartic acid (Asp), which suggests that LYSP could regulate effectively the disorderly metabolized monoamine and amino acid neurotransmitters and improve the learning and memory abilities. Astragalus polysaccharide is also report to down-regulate the extracellular concentration of Glu, glutamine (Gln), glycine (Gly) and taurine (Tau) in the hippocampus of rats with vascular dementia and to enhance the ability of spatial learning and memory [96].

Synaptic damage is one of the AD characteristics in the early stage. Ganoderma lucidum polysaccharide (GLP) could increase greatly the synaptophysin expression and numerica density and surface density in hippocampus, as well as could decrease the average delitescence of rats in Morris water maze, indicating the improvement of learning ability [80].

Some polysaccharides could provide protection and nutrition for neurons. Sulfated polysaccharides from brown seaweeds GS201 enhances significantly the neuronal survival of both hippocampus and neurocortex in a dose-dependent mode, indicating its neurotrophic activity [97].

In vivo models

In vivo study is important to understand AD pathogenesis and progress. *In vivo* models include animal models in preclinical research and models in clinical research.

First of all, animal models of a variety of species have been developed, including worm, rodents, cats, dogs, polar bears, goats, sheep, and nonhuman primate species [98]. Because of strong fertility, short life span, and low cost, mice and rats are the most popular experimental models. AD animal models are classified into induced and spontaneous models. Induced models include transgenic and non-transgenic models. Models featuring amyloid pathology, cholinergic dysfunction, Tau pathology and models showing other features of AD, such as aluminum, scopolamine and environmental factors, have been characterized and employed [99-100]. However, related reports of polysaccharide are limited and the mechanisms need to be studied further.

Huang has used Y-water maze and Morris maze tests to study the learning and memory ability of different models such as scopolamine, 40% alcohol, D-galactose, and senescence accelerated mouse prone strain/8 (SAMP8). The results show that all kinds of dementia model are significantly impaired in learning and memory abilities, while LYSP could improves the abilities [101]. Zibu Piyin Recipe (ZBPYR), derived from Zicheng Decoction, a traditional Chinese medicine formula recorded in the book of Bujuji, written by WU Cheng in the Qing dynasty, is found to improve patients' memory and intelligence in clinic [102]. Effects of different fractions from ZBPYR on scopolamine-induced learning and memory impairment in the mice are investigated. The results show that treatment with polysaccharide and volatile oil result in a significantly shorter escape latency time and swimming distance in the Morris water maze test, as well as shorter latency to cross platform location and increased numbers of location crosses, demonstrating that polysaccharides and volatile oil in ZBPYR exert ameliorating effect on scopolamine-induced

memory dysfunction [103].

The models in clinical research of AD used in TCM study will be discussed briefly in following sections. For no carbohydrate-based drug has been reported in clinic, we take huperzine A (Hup A), instead of polysaccharide as an example [104-106]. Huperzine A is the only launched TCM drug for AD treatment at present [105]. It is an alkaloid isolated from TCM *Huperzia serrata* and acts as an acetylcholinesterase (AchE) inhibitor [104]. In all published clinical trials, there are three modes: randomized placebo-controlled trial, randomized positive controlled trial, and combination treatment trial of Hup A for AD treatment. After oral administration of different scale of Hup A for 12−36 weeks, the efficacy of Hup A is evaluated with different evaluation index, such as mini-mental state examination (MMSE), activities of daily living scale (ADL), Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-Cog), and clinical dementia scale (CDR) [104].

In the model of randomized placebo-controlled trial, effect of Hup A is compared with placebo [104]. In randomized positive controlled trial, study is carried out to compare the clinical efficacy and safety of Hup A with that of positive control, such as acupuncture, Salvia tablet, Vitamin E, Nimodipine, and piracetam [104]. In combination treatment trials, the efficacy is compared between treatment with Hup A alone and treatment with Hup A together with some other AD drugs, such as Ergotamine, Aspirin enteric-coated tablets, and nilestriol [104].

Clinical study for AD

AD drugs are divided into 3 categories by Jeffrey Cummings: disease-modifying immunotherapy, disease- modifying small molecules, and symptomatic agents [107]. Based on mechanism of action, AD treatments consist of amyloid-related therapy, Tau-related therapy, and others [108-109].

Amyloid-based drugs target beta amyloid antagonistic action, including inhibition of Aβ production and aggregation, as well as clearance of Aβ [109]. Tau-based drugs in clinical trials consist of 4 categories: 1), inhibitors of protein kinase such as glycogen synthase kinase-3 (GSK-3) and cyclin dependent kinase-5 (CDK-5); 2), inhibitors of Tau aggregation; 3), microtubule stabilizing agents; and 4), immunotherapy of tau protein [109]. Other drugs target neurotransmitter, antioxidant, anti-inflammatory, and stimulation of nerve growth factor [109]. More details can be referred to the related reviews on clinical trials in AD [108-109].

According to the database of Cortellis, there are 187 drugs in clinical trials in the AD field: 32 drugs on phase III clinical trial, 69 drugs on phase II and 86 drugs on phase I [110]. Four drugs are from TCM, of which three is Huperzine A as AchE inhibitor and one is HSH-971 as β-amyloid antagonist [110]. HSH-971 (also known as GV-971, sodium oligomannurarate), a marine sulfated oligosaccharide from Shanghai Green Valley, is the only carbohydrate-based drug from TCM [110]. A phase III trial was initiated in China in 2014 and recruitment was ongoing in 2016. However, to the best of our knowledge, no polysaccharide clinical drugs on AD have been reported so far.

Future prospects

First, in the development of carbohydrate-based drug for AD, the key problems are difficulties in purification and synthesis of polysaccharide, high complexity of structure, and lack of target study. Because of these problems, the studies of polysaccharide-based drugs lag behind that of small molecules and that of some other macromolecules. In the aforementioned study shown in Table 1, overwhelming majority of studies are conducted with crude polysaccharide. The complex matrices put forward a great challenge for the mechanistic study and quality control. Fortunately, with the rapid development of separation, analytical and synthetic techniques, more and more homogeneous polysaccharides emerge. Then different models (*in vivo* and *in vitro*) are encouraged for screening. Mechanistic study, especially the target study, will be conducted for compounds with positive results. Therefore, work is needed to develop the methodology to efficiently get homogenous polysaccharides and elucidate their structures.

Second, in clinical research, it is a strategy to develop multi-targets and cocktail drugs. A good example is Namzaric capsule, a new drug approved by FDA in 2014 and used to treat moderate to severe dementia of AD [111]. Namzaric capsule contains memantine, a NMDA receptor antagonist, and donepezil, a reversible inhibitor of AchE [112]. TCM puts emphasis on holism and dialectics all the time and is characteristic in multi-pathway, multi-targets and multi-levels in the treatment. Furthermore, because of its highly complicated structure, polysaccharides play a role in AD treatment by multi-pathways. For example, LYSP has anti-AD effect by acting on metabolism of free radicals, cholinergic, monoamine and amino acid neurotransmitter, APP and Aβ expression as well as by anti-apoptosis.

Last, but not the least, clinical research should be paid more attention. As we know, the efficacy and safety of drug are crucial in the drug development. Some TCM have been proven to be efficient in clinic in thousands of years. However, their mechanism might be not clearly enough for the sake of the complex matrices, especially TCM formula. Over-emphasis of preclinical research might lead to the loss of opportunity to develop good drug candidate. Therefore, great efforts are needed in the development of pharmacological methodology for TCM and policy of the approval of TCM-based drug could be adjusted to be consistent with international standards.

Conclusion

AD is a chronic neurodegenerative disease and the mechanism is poorly understood. Its pathological process is complicated and affected by multiple factors. Drugs for AD treatment on the current market mostly target on single molecules and temporarily improve symptoms. However, no drugs

Table 1 TCM polysaccharides and their effects on AD

Name	Source	Effect	Mechanism	reference
Angelica polysaccharide (AP)	*Angelica sinensis* (Oliv.) Diels	Enhanced SOD and telomerase activity	Targeting oxidative stress	[55]
		Increased Bcl-2 and decreased Bax gene expression	Targeting anti-apoptosis	[74]
		Inhibit AchE activity	Targeting cholinergic neurotransmitter	[41]
		Up-regulating Ca^{2+}-ATPase activity and down-regulated Ca^{2+} content	Targeting calcium channel	[41]
Astragalus polysaccharide (APS)	*Astragalus membranaceus* (Fisch.) Bge	Decrease Aβ content	Target Aβ	[22]
		Down-regulate Glu, Gln, Gly and Tau concentration	Targeting neurotransmitter	[96]
		Enhancing AchE activity and decreasing the Ach content	Targeting cholinergic neurotransmitter	[44]
Aqueous extract	*Verbena officinalis* Linn. (Verbenaceae)	Activated PKR and JNK stress kinases	Targeting anti-apoptosis	[88]
CDPS	*Cistanche deserticola* Y. C. Ma	Increased AchE content and ChAT activity	Targeting cholinergic neurotransmitter	[42]
		Increase SOD activity, lower MDA, NO and ROS content	Targeting oxidative stress	[59]
		Upregulated Bcl-2 and down-regulated Caspase-3 expression	Targeting anti-apoptosis	[81]
Cornus officinalis polysaccharide	*Cornus officinalis* Sieb. et Zucc.	Inhibit overexpression of GSK-3β, p-tau(Ser422) and p-tau(Ser396)	Target Tau proteins	[32-33]
Decoctum	*Acorus tatarinowii* Schott	Decreased NOS activity	Targeting oxidative stress	[62]
EJL Polysaccharide	*Lycium barbarum* and *Lycium polygonatum*	Up-regulated Bcl-2 and down-regulated Bax	Targeting anti-apoptosis	[85]
FBD	*Danggui Shaoyao San*, compound formula	Raised the SOD activity and reduce d MDA content	Targeting oxidative stress	[64]
FPS	See weed	Augmented ChAT activity and inhibited AchE activity	Targeting cholinergic neurotransmitter	[43]
GLP	*Ganoderma lucidum* (Leyss. Ex Fr.) Karst.	Increase SOD activity, lower MDA content	Targeting oxidative stress	[60,61]
		Down-regulated c-fos, caspase-3 and fasL gene expression	Targeting anti-apoptosis	[77-80]
		Increase the synaptophysin expression and numerica density and surface density	Targeting synaptic damage	[80]
GLPP	*Ganoderma lucidum* (Leyss. Ex Fr.) Karst.	Decrease Aβ content	Target Aβ	[21]
		Inhibit p-tau Ser396/Ser404 and p-tau Ser199/Ser202 expression	Target Tau proteins	[21]
GS201	Brown seaweeds	Enhanced the neuronal survival	Neuron protection and nutrition	[97]
J2, J3 and J4	*Nerium indicum* Mill. (Apocynaceae)	Activation of Akt survival signaling pathway	Targeting anti-apoptosis	[70]
J6	*Nerium indicum* Mill. (Apocynaceae)	Inactivation of JNK signaling pathway	Targeting anti-apoptosis	[71]
Kadsura heteroclite polysaccharide	*Kadsura heteroclite*	Inhibit Aβ production	Target Aβ	[18]
LBA	Aqueous extract from *Lycium barbarum* L.	Inhibiting the activation of c-Jun N-terminal kinase (JNK)	Targeting anti-apoptosis	[86]
LBB-I and LBB-II	Alkaline extract of *Lycium barbarum* L.	Stimulation of Akt signaling pathway	Targeting anti-apoptosis	[87]
LJW0F2	*Lonicera japonica* Thunb.	Inhibit $A\beta_{42}$ aggregation, $A\beta_{42}$ secretion and the expression of APP and BACE1	Target Aβ	[14]
LNT	*Lentinula edodes*	Decreased LDH, NO and MDA content and increased SOD activity	Targeting oxidative stress	[63]
LYSP	*Millettia Pulchra Kurz* var. Laxior (Dunn) Z Wei	Inhibited the expression of APP, PS1 and PS2	Target Aβ	[19]
		Enhanced SOD and GSH-Px activities and decreased MDA and NO contents	Targeting oxidative stress	[56]
		Up-regulated NE, DA and 5-HT content and down-regulated Glu and Asp content	Targeting monoamine neurotransmitter	[95]
		Improve the learning and memory abilities	–	[101]
OMO	*Morinda Officinalis* How	Inhibition of the brain neuron apoptosis	Targeting anti-apoptosis	[83]
PFR and PBWF	*Polygonum multiflorum* Thunb. and *Lycium barbarum* L.	Increased SOD and GSH-PX activities , decreased MDA and lipofuscin content	Targeting oxidative stress	[65]

Continued

Name	Source	Effect	Mechanism	reference
Poly-gona-polysaccharose	Polygonati rhizoma	Reduce the deposition of Abeta	Target Aβ	[20]
		Reduce neuron apoptosis	Targeting anti-apoptosis	[82]
Polysaccharides from *Qing Xin Kai Qiao Fang*	Compound formula	Decreased the expression of Bax and Caspase-3	Targeting anti-apoptosis	[16]
Qing Xin Kai Qiao Fang	*Qing Xin Kai Qiao Fang*	Inhibit the expression of APP	Target Aβ	[16-17]
Radix polygoni multi-flori preparata polycose	*Polygonum Multiflorum*-Thunb.	Activate NOS in Ammon's horn, SOD and CAT activities	Targeting oxidative stress	[57-58]
SFPS	*Sargassum fusiforme* (Harv.) Setchel	Increased Bcl-2 and decreased Bax gene Expression	Targeting Anti-apoptosis	[75]
VTP	*Vitis viniferal*	Inhibit APP mRNA expression	Target Aβ	[15]
		Increased Bcl-2 and decreased Bax gene expression	Targeting anti-apoptosis	[76]
Yam polysaccharide	*Dioscorea opposite* Thumb.	Increased vitality of SOD, CAT Mg^{2+}-ATPase, Na^{+}-K^{+}-ATPase, reduced MDA content	Targeting oxidative stress	[52]
YLSP	*Millettia pulchra* Kurz Var laxior (Dumm) Z. Wei	Reduced the abnormal Ser202 phosphorylation	Target Tau proteins	[34]
		Scavenged free radicals	Targeting oxidative stress	[53-54]
		Up-regulated anti-apoptotic gene Bcl-2 and down-regulated pro-apoptotic gene p53, Bax and caspase-3	Targeting anti-apoptosis	[53,72-73]
		Down-regulated Ca^{2+} content	Targeting calcium channel	[94]
ZBPYR polysaccharide	Zibu Piyin Recipe compound formula	Ameliorating effect on scopolamine-induced memory dysfunction	–	[103]

to date can prevent, halt, and reverse AD progression. Interactions between macromolecules in cell are neglected in most cases. TCM puts emphasis on holism and dialectics all the time and is characteristic in multi-pathway, multi-targets and multi-levels in the treatment. For example, LYSP has anti-AD effect by acting on metabolism of free radicals, cholinergic, monoamine and amino acid neurotransmitter, APP and Aβ expression as well as by anti-apoptosis, among others. Many other TCM polysaccharides, such as ginseng poly/oligosaccharides, angelica polysaccharide, polygona polysaccharide, and lyceum barbarum polysaccharides, present significant effects by targeting different molecules and pathways.

Owing to difficulties in the separation and purification, synthesis and complicate structure elucidation, crude polysaccharides are studied in the mechanistic researches, which put forward a great challenge for the mechanistic study and quality control of carbohydrate-based drug in the future. Nevertheless, the research and development of carbohydrate-based drug provides a promising way for the AD treatment and we have a long way to go in polysaccharide acquisition, structure elucidation, and mechanistic study.

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