Mini Review **Open Access**

Is the Combination/Multi-target Therapy a New Promise for Alzheimer's Disease?

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Abbreviations: AD: Alzheimer's Disease; Aβ: Beta Amyloid; P-Tau: Phosphorylated Tau; AChEi: Acetylcholinesterase (AChE) Inhibitor; CDK5: Cyclin-dependent Kinase 5; PP2A: Protein Phosphatase 2A; CaMKII: Ca^{2+}/Calmodulin (CaM)-dependent Protein Kinase II; CaN: Calcineurin; IDE: Insulin Degrading Enzyme; syp: Synaptophysin

Since the worldwide demographic ageing, the prevalence of Alzheimer's disease (AD) will increase dramatically. It is a well known complex and progressive disorder, which not only owing to its complex clinical symptoms including cognitive, non-cognitive (such as hallucinations, delusions, anxiety, marked agitation and associated aggressive behaviour) symptoms [1], but also because it's complicated pathogenesis, like multiple genetic factors, oxidative stress, neuroinflammation, and vascular harms, mitochondrial dysfunction, increased amyloid and tau deposition, decreased neurotrophic factors and loss of synapses as well as neurons [2].

Although the detailed mechanism of AD has been investigated for decades, most of those laboratory results still have a long way to transform into clinical use. Currently approved pharmacotherapy only include two categories, the cholinesterase inhibitors and N-methyl D-aspartate receptor (NMDAR) antagonist [3]. They are widely used in clinic, but have been proved to be of limited efficacy and are generally considered to improve or stabilize symptoms rather than affect the underlying disease processes [4]. Thus, many pharmaceutical companies began to seek disease-modifying treatments for AD [2]. Since the important roles of Aβ and Tau accumulation in AD pathogenesis, many potential treatments targeting the accumulation of either amyloid-beta (Aβ) or tau proteins are still under ongoing investigations [5].

Humanized Anti-Aβ antibodies with high and accurate binding properties to Aβ, like the bapineuzumab and solanezumab, was a hot and promising therapeutic strategy in reducing brain Aβ via increasing its clearance [6-11]. In both preclinical studies with transgenic mice that overproduced Aβ and phase 2 clinical studies with mild-to-moderate AD patients, they showed good results in slowing Aβ deposition or reducing the amount of Aβ in the brain. However, negative results as assessed by primary outcomes in both two medicines' phase 3 trials in treating mild-to-moderate AD were released [12,13]. The failed primary end points may in certain way indicate that targeting Aβ alone is not a reliable therapeutic strategy for the treatment of such complex and complicated disease in late stage. Combination/multi-target therapy targeting the accumulation of both amyloid and tau proteins and other facets of neuro degeneration may therefore become a reasonable strategy.

Thus combining medicines with different and/or complementary mechanisms of effects or using multi-target therapy targeting several steps in the neurodegenerative process of AD is a hot topic now. There are many evidences support the combination use of cholinesterase inhibitors and NMDAR antagonist. First, initial evidence from pharmacokinetic and pharmacodynamic data in healthy volunteers showed that memantine and donepezil may be safely used in combination [14]; Second, although there are controversies, some evidences from either clinical trial or systemic review indicate the combination use offer additional benefits to the patient [15-18]. Compare to moderate to severe AD patients receiving mono stable donepezil, similar patients receiving memantine (10 mg twice daily) and same dosage donepezil showed significant benefits in all four main symptom domains of AD, namely, cognition, function, behaviour, and global status [15]. Combined treatment with memantine and AChEIs was effective in patients with AD, particularly in slowing cognitive impairment and preventing the onset of agitation and aggression in elderly AD patients [19]. This may be explained by the fact of preclinical data showed these two drugs act via two different, but interconnected, pathological pathways, and that their complementary activity may produce greater effects than either drug individually [20]. However, there was also trial proved no significant benefits of the combination of donepezil and memantine over donepezil alone [21].

With the combination of behavior symptoms occur in different stages of AD patient, cognitive treatment also commonly used in the combination of non-cognitive treatments, like antipsychotic (AP) or antidepressant (AD) treatments. Evidences indicate that ADs may be reasonable pharmacological alternatives to APs in clinical management of such behavioral symptoms in AD [22]. However, there are no more detailed evidences of such combination therapy. And misuse of sedative medicines in AD patients may cause rapid deterioration. On the contrary, A preliminary open-label trial conducted in Japan suggested that the discontinuation of donepezil treatment in AD patients with behavior symptoms may produce superior efficacy and may make it possible to not increase the dosage of other psychotropic drugs [23]. And there were also evidences suggest that antipsychotic treatment may increase diabetes risk in AD patients [24].

So there are still many blanks for us to explore in the use of combination therapy. Beside the combination of two mono-target medicine, multi-target medicine or complex-component medicine may also provide some interesting clues in the treatment of AD patients. Herbal medicine or Chinese herbal formula with complex-component character may just fit into such strategy. However, few recognition has been paid to herbs in treating AD, except galanthamine and huperzine A [25,26]. Recognitions are even less in the concern of Chinese herbal formula, a combination of several herbs according to Chinese medicine theory. While, there are some very intriguing evidences about AD symptom-improving, or even disease-modifying effects of single herbs

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and herbal formulas in preclinical experiments and clinical trials. The following are some clinical or experimental evidences of herbal medicine in treating AD.

"Ba Wei Di Huang Wan", a very famous Chinese herbal formula, has been reported in significantly improve Mini-Mental State Examination (MMSE) and Barthel Index of patients with AD and cerebrovascular disease [27]. "Yi-Gan San", another traditional herbal medicine, may also significantly improve Neuropsychiatric Inventory (NPI) and Barthel Index in dementia patients [28]. Another Chinese herbal formula named GAPT, also called as GEPT in our previous papers, also showed promising effects in both preclinical and clinical studies on treating AD.

GAPT, a combination of herbal extracts, consists of eight active components pro rata of ginsenoside from ginseng, volatile oil and asarone from acorus tatarinowii schott, flavonoid glycoside from epimedium, tenuifolin from polygala, curcumine from tuber curcuma and others. Preliminary clinical studies of GAPT in treating amnestic mild cognitive impairment showed that it can significantly improved the cognitive function of patients with early-stage AD, amnestic mild cognitive impairment (MCI) [29,30]. In animal studies, GAPT extract can markedly enhance learning and memory of AD rat models induced by hippocampal injection of Aβ1-42 peptide or intravenous injection of Aβ1-40 peptide or STZ, as well as APPV717I transgenic mice and APPswe/PS1dE9 transgenic mice [31-34]. Evidences showed that

GAPT may inhibit γ-secretase (presenilin-1) and promote insulin degrading enzyme and neprilysin, thus reduce Aβ levels in the brain of APPV717I transgenic mice. It may also attenuate the abnormal hyperphosphorylation of Tau protein in hippocampal neurons of APPV717I transgenic mice via inhibiting the expression of Cyclin-dependent kinase 5 (CDK5) and activating the expression of protein phosphatase 2A (PP2A). GAPT also showed significant effectiveness in synaptic protection either before or after the formation of amyloid plaques inAPPV717I mice, which is exerted partially through activating the expression of Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (CaMKII) and synaptophysin, as well as inhibiting the expression of Ca^{2+}/Calmodulin-dependent protein phosphatase 2B (calcineurin, CaN). CaMKII and CaN have been found to play important roles in memory processes and neuronal degeneration. It also showed significant preventive and therapeutic effects in repairing neurons structure, increasing synapses number, as well as restoring balance of Bcl-2/Bax in the hippocampus of APPswe/PS1dE9 transgenic mice. Bcl-2/Bax ratio has well proved as important balance in apoptosis signaling pathway. Above all, GAPT showed significant promise in restoring the dynamic balance state of Aβ and hyperphosphorylated Tau metabolism via targeting multiple-targets (Figure 1).

There are also many interesting clues in the field of single herbs and extracts or derivative from Chinese herbs. For example, curcumin, *Ginkgo biloba* and Ginsenosides. All extracts exert a broad range of
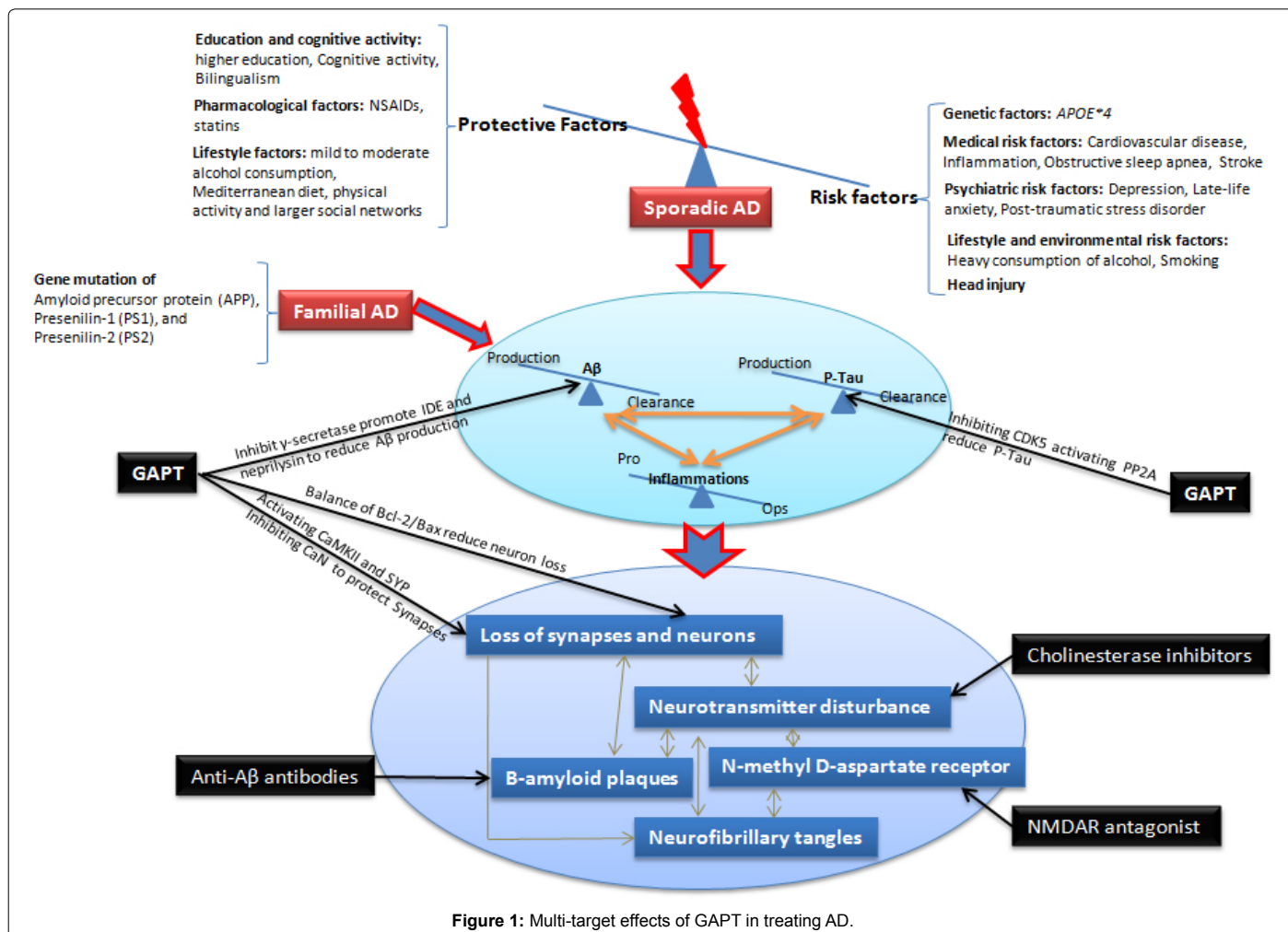

Figure 1: Multi-target effects of GAPT in treating AD.

bioactivity and neuroprotective effects. Curcumin is a well-known food additive in Indian cuisine and is used widely in both Ayurvedic medicine and Chinese medicine. In a 4 weeks randomized, double-blind, placebo-controlled human trial, curcumin (400 mg) has been shown to significantly improve working memory and mood after treatment [35]. In animal studies of curcumin in APPswe/PS1dE9 double transgenic AD mice also showed improved spatial learning and memory ability [36,37]. Such effects may conducted through reducing $A\beta40$, $A\beta42$, and aggregation of $A\beta$-derived diffusible ligands in the mouse hippocampal CA1 area, increase the expression of synapse-related proteins PSD95 and Shank1 and improve structure and plasticity of synapse. Ginkgo biloba is among the most widely used complementary alternative medicines for preservation of cognitive health in aging [38]. The effect in AD treatment include preventing free radical-induced damage, increasing intracellular levels of antioxidant enzymes and restoring calcium homeostasis, as well as attenuating neuron apoptosis, inhibiting membrane lipid peroxidation, anti-inflammatory and direct inhibiting $A\beta$ aggregation [39]. Although previous perspective clinical trial indicate that Ginkgo biloba 120 mg twice a day was not effective in reducing the incidence of AD or all-cause dementia in subjects without dementia at baseline [40], a recent systematic review and meta-analysis of randomized placebo-controlled trials on Ginkgo biloba extract EGb 761® evidenced the clinical efficacy, safety, and tolerability of Ginkgo biloba extract EGb 761 at daily doses of 240 mg in the treatment of patients with dementia [41]. Panax ginsengis another commonly used herbal medicine for the treatment of weakness and fatigue in Asia for thousands of years. Its main active components are ginsenosides, which also have a variety of benefits, including anti-inflammatory, antioxidant, and neuroprotective effect [42]. Ginsenosides have showed effectiveness on AD in vitro and in vivo models [43-45]. An open-label investigation about Panax ginseng in AD patients indicate that 12 weeks ginseng administrition improved the cognitive performance scales according to mini-mental state examination (MMSE) and Alzheimer disease assessment scale (ADAS), and the improved function disappeared after the ginseng withdrawal [46].

Conclusion

Above all, there are evidences of both single herbs and herbal formulas in improving AD symptoms, even in modifying disease effects in clinical trials or preclinical experiments. And many preclinical experiments showed such effects are exerted through a multi-target way. Since the pathogenesis of AD is a complex process involving so many signaling pathways, and development of effective mono-target disease-modifying drugs has been proven to be a difficult task. Therefore, these encouraging preclinical and clinical results may well suggest that multi-target effect of herbal extracts is a new promise for Alzheimer's disease. And the multi-component and multi-target characteristics of Chinese herbs may well be considered as a new promise for treating the complex AD in different stages.

Acknowledgement

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