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Review Article

Tibetan medicine "RNSP" in treatment of Alzheimer disease

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Abstract: Alzheimer disease (Alzheimer Disease, AD) is one of the most common type in senile dementia. Its main pathological features were that a large number of senile plaques gathered in brain extracellular and tangles fibrosis appeared in nerve cells. Currently, the pathogenesis of AD is still uncertain, and scale investigation and combined brain CT, MRI data were analyzed mainly for clinical diagnosis. Mitigation and improvement of the nervous system activity to interfere with the subsequent behavior of the patients are the main methods for treatment. In clinical no drug can really prevent and cure AD. From the view point of Tibetan medicine studies, Tibetan medicine RNSP has effect on improving memory and repairing the neurons in the brain. In this study, we combined the characteristics of AD pathology, pathogenesis, diagnosis and treatment methods to explore the feasibility of Tibetan medicine RNSP for the treatment of AD to provide new ideas for the diagnosis and treatment of AD.

Keywords: Alzheimer disease (AD), Tibetan medicine RNSP, Aβ protein

Introduction

Alzheimer disease (Alzheimer disease, AD) is one of the most common type in senile dementia. In 1907 the German psychiatrist and physician Alois Alzheimer firstly reported the disease [1]. "Lancet" reported latest epidemiological survey of AD and it showed that 2420 million dementia people in worldwide and 4.6 million are new cases until 2005. About 70% of cases are due to AD, and the number of AD patients is expected to be double every 20 years. According to "nature" in 2007, which was a hundred years since the foundation of AD, there were 4.5 million AD patients in the United States and the number of growth is constantly increasing over time [2, 3]. Therefore, understand the cause of AD and find new ways for treatment as soon as possible are very urgent in clinical practice.

The development and use of Tibetan medicine enter a new phase as science. Some Tibetan medicine has been reported having a good effect for age-related diseases, such as RNSP. However, how to assess the clinical effectiveness of Tibetan medicine for treatment of AD is still a problem. In this paper, we detailed analyzed the achievements of RNSP in the treatment of AD and its feasibility in the follow-up studies.

Pathology and pathogenesis of AD

"β-Amyloid" hypothesis theory is important in the pathogenesis of AD. Although the pathogenesis of AD is still not sure, in the 1980's, it was initially reported a major protein component named Aβ [4] can be extracted in senile plaques and then the hereinafter "β-amyloid" hypothesis generated. Aβ protein is originated from single transmembrane β-former protein (Amyloid precursory peptide, called APP), through β and γ-secretase enzyme and were cut into 39 to 43 amino acid peptides. Aβ1-42 only accounts for 10% but it plays a major role in toxicity [5]. It is widely believed that Aβ protein oligomerization is the main cause of AD onset. In the past decades, a large number of studies suggested that Aβ oligomers toxicity mechanism is the main direct cause of AD [5]. The content of the cerebrospinal fluid Aβ42 is treated as a biomarker for detection [6].

Onset of AD caused by other mechanisms: (1) metal ions can cause AD, and aluminum elements can lead to the formation of NFTs and

senile plaques. Cu^{2+} and Zn^{2+} can accelerate the accumulation of Aβ in vivo [7]; (2) Cholinergic damage theory Cholinergic neurons in brain are reduced in AD patients, which reduces the release of acetylcholine (Ach) and serious impediment to the brain's memory and cognitive function. This theory is a most recognized theory for AD pathogenesis in clinical, which is an important foundation for AD therapy and drug development that could be relied on [8]. (3) Excitatory amino acid toxicity theory Studies have shown that glutamate and its receptors are involved in neuronal excited synaptic transmission. A lot of glutamate releasing will cause brain damage and glutamate hyperthyroidism in AD brain. Thereby it causes neuronal damage and results in a series of cognitive impairment in AD symptoms [9]. (4) Tau protein theory indicates that Tau protein is a microtubule-associated protein with highest concentrations. In the AD patients' brain, Tau protein is hyper phosphorylated and lost the role to promote cytoskeletal stability, resulting in NFTs [10]. (5) Other possible pathogenesis Inflammation and immune dysfunction, excessive free radicals and oxidative stress, insulin-related abnormal glucose metabolism and calcium homeostasis, lipid metabolism and changes of mitochondrial enzyme [11] may lead to the occurrence of AD.

The diagnostic criteria for AD

The types of dementia vary in the elders, among which AD is most common and accounts for 60-80% of all senile dementia. Other common types include vascular dementia (incidence second to AD), Lewy Body dementia, mixed dementia, frontotemporal dementia, Parkinson disease, Creutzfeldt-Jakob disease, primary progressive aphasia, normal pressure hydrocephalus, etc. Dementia has various types. And in clinical process, criteria are needed to decide whether the patient has dementia, then we determine whether the dementia is AD. During the past years, Scale was taken for digital scanning like neurophysiological assessment, with clinical accessory examination taken into consideration.

Neurophysiology assessment

Diagnostic and Statistical Manual of Mental Disorders-IV and Criteria of NINCDS-ADRDA were widely used in the world during these years.

In China, MMSE (mini-mental state examination) is the most commonly used tool for assessment. MMSE only takes a few minutes, which makes it easily accepted by the elders. It is mainly used in the evaluation of patients' cognitive function and can detect the degree of intelligence impairment. The cutoff score for cognitive impairment is set according to the degree of education: ≤17 if the patient is illiterate; ≤20 if the patient has primary school education; ≤22 if the patient has high school education; ≤23 if the patient has a bachelor degree or above. Further neuropsychological tests, including memory, executive function, language using, visual-spatial ability and other functional ability tests should be performed. ADL (Activities of daily living) helps to evaluate the impairment of AD patients' daily life ability. BEHAVE-AD, NPI, CMAI (Cohen-Mansfield Agitation Inventory) and other scales are used in the assessment of patients' behavior and psychiatric symptoms.

Clinical detection methods and the application of biomarkers

DSM-IV and NINCDS-ADRDA were regarded as 'gold standard' in the diagnosis of AD. The 'two step method' used earlier could no longer meet the need of diagnosis in the past decades as the rapid development of science and technology. Clinicians gradually noticed that some non-AD patients met the 'clinically probable AD' standard of NINCDS-ADRDA. This may reduce the operability of NINCDS-ADRDA. In 2007, Dubois, Scheltens, and other 13 AD researchers proposed new diagnostic criteria, which conformed to the latest AD researches and was used for early intervention of AD. This criteria stressed the application of biomarkers, including medial temporal lobe atrophy on MRI, decrease of blood supply to temporal and parietal lobe in PET and abnormality of Aβ and tau protein level in cerebral spinal fluid (CSF). These all provided possibilities for the early diagnosis of AD. Clinical detection methods are shown in the **Table 1**.

Treatments of AD

For the treatments of AD, prevention is the main method currently, and psychotherapy is assisted. Drug therapy approach is developed actively. Since the etiology and pathogenesis of AD is unclear, there is no specific way to prevent and reverse progression of the disease.

Table 1. Clinical detection methods and biomarkers

Detection methods	Markers/characters
Structural Imaging (thin layer CT scan and coronal MRI)	Mainly used in excluding diseases other than AD and identifying AD-specific imaging character. e.g., prominent cerebral cortex atrophy with the most obvious atrophy in hippocampal gyrus and medial temporal lobe could be shown, supporting the diagnosis of AD
Functional Neuroimaging(positron emission tomography (PET) and single photon emission computed tomography (SPECT)	18-fluorodeoxyglucose PET (FDG-PET) was mainly used in measuring the decrease of glucose metabolism in temporal lobe, parietal lobe, posterior cingulate cortex and precuneus, which revealed the degree of glucose metabolism decrease
Electroencephalogram (EEG)	Low specificity in AD detection. Decreased α wave, increased θ amplitude and decreased average frequency aided the differential diagnosis of AD
CSF works (Aβ, Tau protein)	Decreased Aβ42 level, increased tau-protein and phosphate tau-protein level were found in AD patients' CSF. The sensitivity and specificity of the method were relatively high

The existing drugs are usually used to improve the patient's actions and behavior. Common psychological therapy and development of drugs are as follows:

Psychotherapy

Intervention and treatment have certain effects for patients in the early stage, including the use of drugs to improve cognitive function and psychiatric symptoms. While psychosocial treatment and good nursing care can slow the progression of disease to some degree. For patients with advanced disease, care should be given in assisting their lives, but not completely arranged. Try to make the patient self-care, such as washing, eating, walking and so on. We cannot allow them go out alone to avoid lost or falls. Various utensils and household items should be taken good care of in order to prevent accidental injury and injury others [12]. Currently psychotherapy can only alleviate AD, but it cannot be used as a mean of healing.

Aβ protein reduction and clearance targeted drug development

Aβ protein oligomers are considered to be the main causes of AD. In clinical, a number of drugs that targeted for Aβ are considered to use and study for prevention and treatment of AD. The main categories are as follows: (1) The protease inhibitor small molecules can inhibit the secretion of the enzyme β- and γ-secretase; (2) Try to get suppressed Aβ oligomers, and accelerate its clearance of drugs; (3) For Aβ aggregation process produced inflammation, develop some anti-inflammatory drugs; (4) Cholesterol-lowering drugs are used to lower cholesterol levels in the brain, such as statins drugs; (5) Cu^{2+} and Zn^{2+} chelator antibiotic clioquinol (clioquinol) are used, which kind of approach has now entered the clinical phase; (6) Reducing synaptic function and neuronal deteriorated effects which are brought by Aβ amyloid, such as anti-oxidants and increasing neurotrophic drugs [13]. Despite a series of measures come up, these drugs are still in the pilot study stage.

Interventional neuromodulator targeted drugs

In AD pathogenesis, cholinergic damage theory and excitatory amino acid toxicity theory have already been mentioned as the major causes of AD, which are the direct interventional targets in clinical. The approved drugs for the treatment of AD are mainly the two categories including the cholinesterase inhibitors and glutamate receptor antagonists:

(1) Cholinesterase inhibitors: The efficiency of cholinesterase inhibitor has been widely recognized in improving AD patients' symptoms. Cholinesterase inhibiting drugs which are commonly used including the following: donepezil (donepezil), Rivastigmine (rivastigmine) and galantamine (galantamine). Under normal circumstances, the cholinesterase inhibitor is well tolerated. Side effects are common in the gastrointestinal such as nausea, diarrhea and vomiting [8].

(2) Glutamate receptor antagonists: brain tissue damage caused by the massive release of

glutamate would lead to the overactive glutamate in AD brain, resulting in neuronal damage and AD symptoms such as a series of cognitive impairment, which is the basis for generating glutamate drug; hydrochloride memantine is N-methyl-aspartate (NMDA) receptor activator, which has also been approved for AD [9].

(3) Application of other drugs: carbamazepine, antidepressants and other psychiatric drugs can be used as emotional stabilizers, but these drugs often have strong side effects, so are not suitable for widespread use. Vitamin E, Ginkgo biloba extracts, Huperzine A and pentoxifylline have also been reported in treating AD, but lacking clinically evidence [14].

To sum up, currently clinical AD drugs mainly focus on easing and improving the AD subsequent behaviors and cannot cure AD.

Feasibility analysis of Tibetan RNSP in the treatment of AD

Currently, in the world, AD incidence is gradually increasing, and the number of patients is rapidly growing, and the cost of caring for AD patients is also increasing. Those have become worldwide problems; particularly the use of new drugs in the course of treatment which can better prevent and treat AD has become the focus of attention in this field. In accordance with the "three", currently clinical drugs can only play a role of remission and improvement, and it is difficult to prevent and treat AD in advance. Therefore, the research and development of drugs for AD are not only great challenges but also new opportunities; to seek and develop new drugs will be one of the most important ways to treat AD. Tibetan medicine has gradually been recognized in thousands of years as a "mysterious" medical drug; on this premise, some Tibetan medicines were considered to be with a potency to treat AD, such as: 25 flavor pearl pills for comprehensive treatment of vascular dementia [15], salidroside for the treatment of AD mouse model [16], of which RNSP had the strongest relation with AD; someone has conducted a series of experiments to explore RNSP efficacy in the treatment of AD.

Tibetan characteristics and related studies

For a long time, through practice, development and exploration, Tibetan medicine theory has already formed an independent medical system, and Tibetan medicine has also become an ethnic cultural deposition in thousands of years. With the development and progress of times, Tibetan medicine which is full of mystery has been acknowledged and understood by the region outside of Tibetan and its validity is gradually being recognized; Japan, the United States, Germany and other countries also have a great interest on the Tibetan medicine; in the treatment of many difficult miscellaneous diseases which cannot be cured by general medicines, Tibet drugs could be selectively used. For example: Ruyi zhenbao pill and Ershiwuwei donkey blood pills can be used to treat rheumatoid arthritis, ankylosing spondylitis and rheumatoid arthritis; 5 flavors musk pills could be used for pain, neuralgia and dispelling wind [17]. However, the composition of Tibetan medicine is very complex, and its active ingredient is not clear, so it is difficult to obtain quantitative research and application and it is difficult to grasp its treatment mechanism in clinical, which prevents the development and promotion of Tibetan medicine to some extent. At present, the common method for Tibetan medicine research is to select the active ingredients of at least two herbs as the index components of quality control and determine their content to improve quality standards, eventually rising to the understanding of the mechanism [18].

Tibetan medicine for the treatment of age-related diseases providing a basis for the treatment of AD

There are many reports on Tibetan medicine for the treatment of many age-related diseases in clinical, such as: fifteen smell incense scatter, fifteen smell Longdanhua scatter for treatment of senile chronic bronchitis, the effect of Nuodikang capsule on plasma coagulation in elderly patients with acute cerebral infarction, Nuodikang capsule for treatment of senile coronary heart disease combined migraine pain, Rhodiola and spearheading for treatment of depression in elderly patients. Tibetan medicine has a significant effect on age-related diseases in these reports. This shows that the Tibetan medicine have certain effects and advantages for the treatment of age-related diseases. According to this idea, it is easy to imagine there is likely some of Tibetan medicine may play a role in senile dementia.

Therefore, some reported that Tibetan medicine had a certain effects on Alzheimer disease, and some Tibetan medicine had the therapeutic potential for AD. For example: 25 flavor pearl balls for comprehensive treatment of vascular dementia [15], Salidroside for the treatment mouse with AD [16]. These experiments are generally tested on animals, but they require a lot of clinical trials.

Therapeutic effects of RNSP for Alzheimer disease

The composition and function of RNSP: Most unique plateau animals, plants and minerals medicine were selected for RNSP, and the black pill are mainly composed of Zuotai, natural pearls, natural bezoar, antelope horn, musk, saffron, sandalwood, benzoin, dalbergia, nine eye stone, agate, coral and other 70 kinds of precious Tibetan medicine. Currently RNSP mainly has the following clinical applications: (1) for the treatment of cerebral vascular diseases; (2) for sedation and anticonvulsant; (3) improve learning and memory; (4) improve neurological function; (5) lower blood pressure. RNSP can improve brain blood vessels, learning and memory effect, so it is easy to be linked for AD treatment. RNSP can reduce generation of Aβ40 and Aβ42 in transgenic model mouse [19], change the rats' cognitive ability, and its efficacy in clinical trials are also partly affirmed [20].

Therapeutic effects of RNSP for animal models of AD

For the effect of RNSP on senile dementia, it is mainly based on animal models. For example: RNSP affects the content of β-endorphin in rats with brain damage. It also affects monoamine neurotransmitters in rats with brain injury. It also improves the mouse's memory-acquirement-disability and the effect of memory-reproduction-missing in mice, etc. These subjects are AD model mouse, the common approach is that to Tg2576 overexpressed Aβ40 and Aβ42 protein in transgenic rats, or perfuse rats' brain. The brain is damaged, and shows the performance of AD symptoms. The mice were administered to RNSP and changes of related protein and mental conditions are observed. Experiments showed that RNSP had positive effects on clearance of Aβ protein and improved memory in rats with AD [19].

However rat model used in drug study has deficiencies in itself. Transgenic (Tg) rodent were used to express mutant human APP and PS gene in a large number of studies. These Tg mice are mainly used for observing the deposition of amyloid plaques, cognitive and neurological dysfunction [21]. It was also used to assess whether a candidate drug may reduce "Aβ burden" [22]. These drugs that used in transferred AD animal models, when transferred back to AD patients, they showed worthless. For example, there are more than 100 kinds of molecular complexes that can reduce Aβ amyloid plaques in transgenic animals, but many complex molecule drugs are completely short of clinical therapeutic and prevention effect of AD. Probably it was because Aβ was highly expressed in AD transgenic mice, which was much faster than that in AD patients. While in sporadic AD patients, many conditions do not meet overexpression standard, therefore it resulted a huge difference between transgenic animal and AD patients [23].

From the current conditions, Tibetan medicine for the treatment of rats, also need to be treated cautiously, and the data which were obtained from the rat cannot be converted directly to humans. In order to verify the therapeutic effect of RNSP, a lot of clinical trials are required to verify.

Clinical trials of RNSP

There are some studies about RNSP clinical trials, but only the scale was used for investigation and analysis. It has certain credibility, but to assess the entire impact of drugs on the AD it is still far from enough.

Then, in RNSP clinical trials process, how to evaluate their effect? The literature on using The scale was used for test of memory and viability in some literatures in order to achieve the purpose of assessment on therapeutic effect. Maybe we can combine with **Table 1** to give the latest diagnostic tools for AD. For example, less trauma and accurate diagnostic rate are the advantages of CSF biomarkers in detecting AD. It can make a prediction for somebody who has AD pathology but not in dementia. That is to identify "preclinical" AD. Prediction of CSF biomarkers Aβ42, total tau and phosphorylated tau has become an essential part for the process of treatment and pro-

gression of AD. If we can confirm that RNSP indeed produces a strong effect on the reduction of Aβ or tau protein, RNSP is very worthy of promotion and improvement for treatment of AD. In addition, we should compare RNSP with other generic drugs. For example, combined with brain MRI, CT data, intelligent screening result, and changes of Aβ oligomers content to assess RNSP for treatment of AD comprehensively, this will provide RNSP with a new basis for the treatment of AD.

If it is certain that RNSP indeed plays a role for the treatment of AD in clinically, then in the later it also involves in the study on mechanisms of treatment. Bulked segregant analysis for qualitative and quantitative analysis on RNSP will also be a huge job. In addition, RNSP contains large amounts of metal ions. In the clinical trials process, we also need to take full account of its negative effects.

In summary, RNSP treatment for AD in early animal and clinical trials, has made some achievements, proving RNSP has a role in reduction and removal of Aβ and improvement on memories for patients with AD. But it also requires a lot of follow-up clinical trials to find a reasonable evaluation standards and systems to apply RNSP in clinical practice.

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Disclosure of conflict of interest

None.

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