Research protocol

Project summary

Alzheimer’s disease (AD) is the most common progressive neurodegenerative disorder in the developed world, and it is estimated that the number of patients with AD will reach 115 million by 2050. Although there is a wide range, the average time from diagnosis to death is 8–10 years. In the present study, we investigated the clinical effects of plasminogen, the key substrate of the fibrinolysis system, in AD. The plasminogen was periodically used in AD patients, and the treatment effects were observed. The clinical study was an open-label, one arm, and non-randomized study. The plasminogen was administrated to the AD patients by intravenous injection for up to 72 weeks. The changes of memory function measured by MMSE scoring system, and safety were investigated during plasminogen treatment. It is expected that the patient's memory function will be improved after plasminogen treatment.

General information

Protocol title: A Study of Plasminogen in Subjects With s Alzheimer's disease (AD)

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Rationale & background information

Alzheimer's disease (AD) is the most common progressive neurodegenerative disorder in the developed world, and it is estimated that the number of patients with AD will reach 115 million by 2050. The prevalence of AD increases with advancing age; its prevalence is 10–30% in individuals over 65 years old, and its incidence at least doubles every 10 years after 60 years of age. Although there is a wide range, the average time from diagnosis to death is 8–10 years [1].

Although the exact mechanism underlying the pathogenesis of AD has not been fully elucidated, it is widely believed that the extracellular accumulation of amyloid beta (Aβ) peptide aggregates and the intracellular accumulation of phosphorylated Tau protein aggregates (neurofibrillary tangles) play key roles in the pathological consequences of AD [2]. These pathological processes cause synaptic degeneration, neuronal death, and consequently, decreased synthesis and release of the neurotransmitter acetylcholine from affected cholinergic neurons. Most of the drugs that are currently approved for

AD treatment merely alleviate symptoms either by increasing acetylcholine levels or by inhibiting acetylcholine catabolism. These drugs are effective in slightly improving the cognitive abilities of AD patients, but they do not offer a cure [3]. In addition, in recent decades, the development of novel AD treatments has primarily focused on treating the pathogenesis of the disease, e.g., preventing the formation of Aβ and Tau aggregates, mainly via blocking antibodies [2]. However, to date, these novel strategies have not yielded convincing results in terms of disease improvements.

The plasminogen activator (PA) system is a general proteolytic system in which an active protease, plasmin, is formed from its parent protein, plasminogen, by one of two physiological PA systems: tissue-type PA (tPA) or urokinase-type PA (uPA). Both tPA and uPA can be inhibited by physiological inhibitor-1 (PAI-1), and excessive plasmin can be inhibited by α2-antiplasmin [4]. The PA system/plasmin is responsible for degrading many extracellular matrix (ECM) proteins, including fibrin, denatured proteins and protein aggregates [5], and this system participates in tissue remodeling and cell migration during neurotoxicity, neuroprotection, and cerebral blood flow [6].

Previous in vitro studies from us and others have suggested that plasminogen/plasmin is involved in activating the latent forms of certain neurotrophic factors, such as pro-nerve growth factor (NGF) and pro-brain-derived neurotrophic factor (BDNF), thus producing the active/matured forms of these factors [7]; these active/matured forms further play key protective roles in regulating the growth, survival, and differentiation of neurons, including cholinergic neurons, and thus provide important neuronal support in AD and other neurodegenerative diseases [8-10].

In addition, some studies have suggested that the PA system is closely related to the progression of AD. In vitro studies have shown that plasmin cleaves and degrades extracellular Aβ1-40 (Aβ40) and Aβ1-42 (Aβ42), preventing their aggregation into β-pleated sheet structures [11]. Plasmin activity is reduced in AD [12]. Furthermore, the chronic systemic administration of recombinant t-PA attenuates AD-related pathology in a transgenic mouse model of AD by reducing the cerebral Aβ levels and improving the cognitive function of the treated mice [13]. In addition, our early preliminary results showed that exogenous plasminogen accumulates at sites of injury, promotes the degradation of misfolded proteins, including alpha-synuclein, transactive response

DNA-binding protein 43 (TDP-43), the Aβ40 peptide, the Aβ42 peptide and the Tau protein, and promotes the repair of nerve injury and dysfunction in neurodegenerative diseases [14-18]. All these studies suggest that the PA system may play important roles in AD. Therefore, in the current study, we used ex vivo and in vivo approaches to investigate the functional roles of plasminogen in AD in both preclinical and clinical studies.

References (of literature cited in preceding sections)

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Study goals and objectives

1. To explore the efficacy of plasminogen in the treatment of AD including the effects on memory function

2. To explore the safety of plasminogen in the treatment of AD including side effect events such as bleeding, hypersensitivity Reactions and infection.

Study design

Study Type: Interventional

Actual Enrollment: 20 participants

Observational Model: Other

Time Perspective: Prospective

Official Title: A Study of Plasminogen in Subjects With Alzheimer’s disease (AD)

Actual Study Start Date: September 05, 2018

Estimated Primary Completion Date: May 2023

Estimated Study Completion Date: October 2023

Inclusion Criteria:

1. Written informed consent/assent obtained prior to any assessment performed

2.Age 50 to 100 years old (including 50 and 100 years old), male or female;

3. Meet the diagnostic criteria of "likely ad dementia" of the National Institute on aging Alzheimer's disease association (NIA-AA) (2011);

4. The subjects are primary school graduates / graduates and above, and have the ability to complete the cognitive ability test and other tests specified in the program;

5. Memory loss lasted for at least 6 months and tended to worsen gradually;

6. Subjects with mild or moderate illness: 0 ≤ total score of MMSE ≤ 26;

7. Total score of Clinical Dementia Rating Scale (CDR):

Mild dementia: CDR = 1.0; Moderate dementia: CDR = 2.0.

Key Exclusion Criteria:

1.Dementia caused by other reasons: vascular dementia, central nervous system infection, Creutzfeldt Jakob disease, Huntington's disease, Parkinson's disease, Lewy body dementia, traumatic dementia, other physical and chemical factors (such as drug poisoning, alcoholism, carbon monoxide poisoning, etc.), important physical diseases (such as hepatic encephalopathy, pulmonary encephalopathy, etc.), intracranial space occupying lesions (such as subdural hematoma, brain tumor), endocrine disorders (such as thyroid disease, parathyroid disease), and vitamin B12, folic acid deficiency or any other known cause;

2.Have suffered from central nervous system diseases (including stroke, optic neuromyelitis, epilepsy, etc.);

3.Subjects who were diagnosed with psychiatric disorders according to DSM-V criteria, including schizophrenia or other mental diseases, bipolar disorder, severe depression or delirium;

4.Abnormal laboratory indexes: liver function (ALT and AST) exceeded 1.5×ULN, renal function (CR) exceeded 1.5×ULN, and creatine kinase exceeded 2×ULN;

5.Untreated hypertensive and hypotensive subjects at screening, or hypertensive subjects with uncontrolled hypertension after treatment; subjects with good blood pressure control after treatment can be determined by the investigator to be suitable for inclusion in this study;

6.Within 1 month of the screening visit, the subject has new or ongoing unstable or serious heart, lung, liver, kidney and hematopoietic diseases according to the judgment of the researcher, and does not meet the conditions for clinical research;

7.Clinically, people with significant allergic reaction history, especially drug allergy history, or known allergy to this product and its excipients;

8.Dyspepsia, esophageal reflux, gastric bleeding or peptic ulcer disease, frequent heartburn (≥ once a week) or any surgical operation that may affect drug absorption (such as partial / total gastrectomy, partial / total small bowel resection and cholecystectomy) within 6 months before screening;

9.Alcohol or drug abusers;

10.Human immunodeficiency virus antibody (ant HIV) and Treponema pallidum antibody (ant TP) are positive;

11.Those who are currently using and cannot stop using drugs for Alzheimer's disease;

12.Female subjects with positive pregnancy test or lactation and subjects unable to take effective contraceptive measures or have family planning;

13. Participated in other clinical trials within 3 months before the screening visit;

14.There are other situations that the researcher believes are not suitable to participate in this study.

Methodology

Brief name： Plasminogen

Provided： The clinical doctors or nursing staff with more than 5 years of clinical work experience administrated the intervention.

The method： The intervention was administrated by an individual face to face.

Place： Based on the condition of patients, the intervention was performed at the home of patients or at Beijing Chang'an Chinese and Western Integrated Medicine Hospital.

Regimen： The clinical study was an open-label, one arm, and non-randomized study.

Treatment duration： 72 weeks

Method of administration： Intravenous injection, at dose of 50-200 mg each time, 1 time per 1-3 days, two weeks as one treatment course, 2 week intervals between courses. Sometimes, Intravenous injection was administrated combined with atomization inhalation. Atomization inhalation:1 time per day, 10 mg each time, beginning on the third day of treatment.

Measurement of outcomes：

Primary Outcome Measure

The memroy function according to the Minimum Mental State Examination (MMSE) was assessed at [at baseline, 2w、6w、10w、22w、46w]

Secondary Outcome Measures

Adverse events measured by [blood routine test, blood biochemistry, coagulation function, hemolysis function, urine routine test, 12 lead ECG, physical examination, vital signs, etc] at [baseline, 22w, 46w]

Safety considerations

WARNINGS AND PRECAUTIONS

Bleeding: plasminogen administration may lead to bleeding at lesionsites or worsen active bleeding. Discontinueplasminogen if seriousbleeding occurs. Monitor patients during and for 4 hours after infusionwhen administering plasminogen to patients with bleeding diathesesand patients taking anticoagulants, antiplatelet drugs, and other agentswhich may interfere with normal coagulation.

Transmission of Infectious Agents: plasmiogen is made from human blood and therefore carries a risk of transmitting infectious agents, e.g.,viruses, the variant

Creutzfeldt-Jakob disease (vCJD) agent, andtheoretically, the Creutzfeldt-Jakob Disease (CJD) agent.

Hypersensitivity Reactions: Hypersensitivity reactions, includinganaphylaxis, may occur with plasminogen. If symptoms occur,discontinue RYPLAZIM and administer appropriate treatment.

Follow-up

All subjects entered the 90 day safety follow-up period after completing the last blood collection and safety inspection of the administration. Since the first administration, they came to the hospital for a safety inspection when they were out of the group or early out of the group at 30 and 90 days, and plasminogen inhibitors and infectious markers were carried out during the screening period and when they were out of the group or early out of the group at 90 days. At the same time, the subjects were interviewed for compliance by telephone/in hospital within 60 days to collect whether there have been adverse events and concomitant medication since the last visit.

Data management and statistical analysis

This study will use EDC system to collect and manage data. The data management process should comply with the Good Clinical Practice (GCP) and the corresponding regulatory requirements for data management, comply with the standard operating procedures (SOP) of the data management department, and ensure the authenticity, accuracy, integrity, reliability and traceability of clinical trial data (EDC system will record all audit trails). The details of data management will be provided in the data management plan, and the responsibilities of researchers, supervisors and data managers will be clarified to ensure the quality level of each stage of data management.

In this study, the Data Manager Associate (DMA) writes the data management plan as a guiding document for the entire data management process. All processes of data management should be operated according to the data management plan, which should be updated according to the specific progress of the project.

The biostatisticians and major researchers shall formulate a statistical analysis plan according to the research scheme, and improve the statistical analysis plan document

before data locking. The frequency and percentage of the classified indicators in the completion of the study, demography and baseline characteristics were calculated, and the numerical indicators were analyzed descriptively.

(1) Efficacy analysis

The measurement data adopts the sample number, mean, standard deviation, median, Q1, Q3, minimum value, maximum value, error bar chart, broken line chart, etc; Counting data included the number of cases, constituent ratio, rate, 95% CI, straight bar chart, percentage constituent chart, etc.

(2) Security analysis

The method of statistical description is mainly used to describe the number and incidence of adverse events/reactions, serious adverse events/reactions, adverse events/reactions leading to drug withdrawal, drug suspension, and withdrawal from the study. According to MedDRA, the medical code was graded to describe the number and incidence of adverse events/reactions, serious adverse events/reactions, adverse events/reactions leading to drug withdrawal, drug suspension, and withdrawal from the study under each system organ classification (SOC) and preferred term (PT). The severity of various adverse events/reactions was evaluated according to NCI-CTCAE (Version 5.0), and the number and incidence of adverse events/reactions in each system were described according to the severity. For laboratory, vital signs, ECG, physical examination and other safety data, the baseline data, post treatment data and post treatment change data will be summarized.

Quality assurance

Before the start of the clinical trial, the researchers should receive the training of the trial plan, so that the researchers can fully understand and understand the clinical trial plan and the specific connotation of each indicator. The quality control personnel shall check the basic conditions of the clinical trial to ensure that the clinical trial conditions can meet the requirements of the scheme. During the trial, the investigator shall carefully carry out the clinical operation and other work according to the requirements of GCP, agency SOP and the trial plan, and make records truthfully, timely, completely

and normatively. The quality control personnel shall check the quality of the test process and the corresponding original records. After the test, the research unit shall sort out the corresponding project documents, which shall be checked by the quality control personnel and archived. The quality assurance department of the clinical research unit shall carry out quality control for the feasibility of the tests carried out. When non conformities are found, the researcher and the person in charge of the unit shall be notified in time to make corrections, and the corrections shall be tracked.

Expected outcomes of the study

The success of this study will provide new treatment methods for Alzheimer's disease patients, reduce treatment costs, bring good news to patients, and reduce the social burden.

Dissemination of results and publication policy

The purpose, content and results of this clinical trial as well as all future information must be strictly confidential. The copyright of all materials and results (including test data and its derivative data) belongs to the sponsor.

Duration of the project

Actual Study Start Date: September 05, 2018

Estimated Study Completion Date: October 2023

Ethics

The patients are minors or infants, so the ethical review is more strict. All personnel responsible for recruitment shall fully introduce the drug situation, benefits and risks during the trial to the patient's guardian. In addition, the patient shall be given adequate financial compensation to obtain informed consent as much as possible.

Informed consent forms

Subjects must give informed consent to participate in the trial before receiving treatment in order to protect their legitimate rights and interests. The investigator has the responsibility to completely and comprehensively introduce the purpose of this study, research methods, drug effects, reasonable expected benefits, possible toxic and side effects and possible risks to the subject or its designated agent. The investigator should let the subject know their rights, risks and benefits to be borne, and should promptly inform the subject of any new information about the drug for the trial. The subjects should be informed that this clinical trial is based on the principle of voluntary participation, and they can withdraw from the trial unconditionally at any time during the trial, and they will not be punished for withdrawing from the trial. The subjects shall be informed that the research party and sponsor have the right to read, save and statistically process the test data of the subjects according to the provisions of relevant laws and regulations. The version, preparation date or modification date of the informed consent shall be indicated. Only subjects who fully understand the risks and benefits of this clinical trial, as well as potential adverse events, and sign their names and dates on the informed consent form can participate in this clinical trial. If the test protocol has been modified to a certain extent during the trial, the informed consent form needs to be modified accordingly according to the modified content, and the informed consent resigned by the subject after being approved by the Ethics Committee.