Research protocol

Project summary

Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease affecting motor neurons and other neuronal cells, leading to severe disability and eventually death from ventilatory failure. Currently, riluzole and edaravone are the only FDA-approved drugs to treat ALS, but they exert modest beneficial effects on survival and disease progression. Thus, the development of effective therapeutic strategies to inhibit disease progression, reverse disease symptoms, or even treat the disease is urgently needed.The plasminogen was periodically used in ALS 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 ALS patients by intravenous injection for up to 72 weeks. The changes of clinical symptoms measured with the Revised Amyotrophic Lateral Sclerosis Functional Rating (ALSFRS-R) were investigated during plasminogen treatment. It is expected that the patient's clinical symptoms will be improved after plasminogen treatment.

General information

Protocol title: A Study of Plasminogen in Subjects With Amyotrophic lateral sclerosis (ALS)

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Rationale & background information

Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease affecting motor neurons and other neuronal cells, leading to severe disability and eventually death from ventilatory failure [1]. Currently, riluzole and edaravone are the only FDA-approved drugs to treat ALS, but they exert modest beneficial effects on survival and disease progression [2]. Thus, the development of effective therapeutic strategies to inhibit disease progression, reverse disease symptoms, or even treat the disease is urgently needed.

Sporadic ALS (sALS) with an unknown cause accounts for more than 90% of ALS cases. Familial ALS (fALS), which is caused by mutations in a number of genes, such as copper-zinc superoxide dismutase (SOD1), transactive response DNA-binding protein 43 (TDP-43), and fused in sarcoma (FUS), accounts for the remaining ALS causes.

The most thoroughly studied forms of fALS, which account for 10-15% of all fALS cases, are caused by mutations in the gene encoding SOD1, an abundant antioxidant enzyme that catalyzes the dismutation of superoxide to hydrogen peroxide (H_2O_2) and dioxygen. The pathological misfolding and aggregation of mutant SOD1 proteins induce numerous toxic effects; hence, reducing the levels of toxic SOD1 mutants has been suggested as a promising therapeutic strategy for SOD1-related ALS [3]. Correspondingly, transgenic mice overexpressing mutant human SOD1 (hSOD1), particularly those with a change from glycine to alanine at codon 93 (SOD1-G93A), have become the benchmark preclinical model for screening ALS therapies [2].

TDP-43 is a DNA/RNA binding protein that has been identified as a key component of insoluble and ubiquitinated inclusions in the neurons of approximately 97% of patients suffering from ALS [4]. Interestingly, intracellular TDP-43 aggregates are observed in SOD1-G93A mice [2, 4].

The plasminogen activator (PA) system is a general proteolytic system in which the active protease plasmin is generated from its parent protein plasminogen by one of 2 physiological PA proteases: tissue-type PA (tPA) or urokinase-type PA (uPA). Both tPA and uPA are inhibited by physiological inhibitor-1 (PAI-1), and excess plasmin is inhibited by α2-antiplasmin [5]. *In vitro* studies have suggested that plasmin is involved in the degradation of extracellular matrix components, including fibrin and some central nervous system (CNS) pathological proteins, including amyloid beta (Aβ) peptide and α-synuclein [5, 6], and in the conversion of the latent forms of certain neurotrophic factors, such as pro-brain-derived neurotrophic factor (pro-BDNF), to their active/mature forms [7], which subsequently play key roles in regulating the growth, survival, and differentiation of motor neurons [8]. In addition, some studies have shown that the PA system is closely related to nerve degeneration and regeneration processes after injury, such as remyelination and neuritogenesis [9]. Furthermore, our early preliminary data suggested that in both non-neurodegenerative and neurodegenerative diseases, including spinal muscular atrophy (SMA), Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), and Huntington's disease (HD), exogenous plasminogen accumulates in the injured area, promotes the degradation of pathological proteins such as fibrin and SOD1, increases tissue regeneration, enhances injury repair, and restores normal functions [10-17].

References (of literature cited in preceding sections)

1. Oskarsson B, Gendron TF, Staff NP. Amyotrophic Lateral Sclerosis: An Update for 2018. Mayo Clin Proc. 2018 Nov; 93 (11):1617-1628.

2. Sever B, Ciftci H, DeMirci H, Sever H, Ocak F, Yulug B, Tateishi H, Tateishi T, Otsuka M, Fujita M, Başak AN. Comprehensive Research on Past and Future Therapeutic Strategies Devoted to Treatment of Amyotrophic Lateral Sclerosis. Int J Mol Sci. 2022 Feb 22;23(5):2400.

3. Abati E, Bresolin N, Comi G, Corti S. Silence superoxide dismutase 1 (SOD1): a promising therapeutic target for amyotrophic lateral sclerosis (ALS). Expert Opin Ther Targets. 2020 Apr; 24 (4):295-310.

4. Cai M, Lee KW, Choi SM, Yang EJ. TDP-43 modification in the hSOD1(G93A) amyotrophic lateral sclerosis mouse model. Neurol Res. 2015 Mar;37(3):253-62

5. Aisina, R. B, Mukhametova, L. I. Structure and function of plasminogen/plasmin system [J]. Russian Journal of Bioorganic Chemistry, 2014, 40(6):590-605.

6. Kim KS, Choi YR, Park J-Y, et al. Proteolytic cleavage of extracellular α-synuclein by plasmin implications for parkinson disease. J Biol Chem 2012;287:24862–72.

7. Lee, P Kermani, K K Teng et al. Regulation of Cell Survival by Secreted Proneurotrophins Science Actions. 2001 Nov 30; 294(5548):1945-8.

8. Beck M, Flachenecker P, Magnus T, Giess R, Reiners K, Toyka KV, Naumann M. Autonomic dysfunction in ALS: a preliminary study on the effects of intrathecal BDNF. Amyotroph Lateral Scler Other Motor Neuron Disord. 2005 Jun;6(2):100-3].

9. Seeds N W, Akison L, Minor K. Role of plasminogen activator in spinal cord remodeling after spinal cord injury [J]. Respiratory Physiology & Neurobiology, 2009, 169(2):141-149.

10. Shen Y, Guo Y, Mikus P, Sulniute R, Wilczynska M, Ny T, Li J. Plasminogen is a key proinflammatory regulator that accelerates the healing of acute and diabetic wounds. Blood. 2012 Jun 14; 119(24):5879-87.

11. Li JN. Method for prevention or treating diabetic mellitus nerve injury and related diseases. US20180369345.

12. Li JN. A method and drug for treating spinal muscular atrophy. US17/924,620.

13. Li JN. A method and drug for treating Parkinson's disease. US17/914,267.
14. Li JN. A method and drug for treating Alzheimer's disease. US17/914,265.
15. Li JN. Method and medicine for preventing and treating multiple sclerosis.US17/797,504.
16. Li JN. A method and a drug for promoting the degradation of misfolded proteins and their aggregates. US17/914,271.
17. Li JN. Method and medicine for treating Huntington's disease. WO2021/190563.

Study goals and objectives

1. To explore the efficacy of plasminogen in the treatment of ALS including the effects on various clinical symptoms

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

Study design

Study Type: Observational

Actual Enrollment: 20 participants

Observational Model: Other

Time Perspective: Prospective

Official Title: A Study of Plasminogen in Subjects With amyotrophic lateral sclerosis (ALS)

Actual Study Start Date: September 05, 2018

Estimated Primary Completion Date: May 2023

Estimated Study Completion Date: October 2023

Inclusion Criteria:

1.Male and female patients ⩾18 years of age with Sporadic or familial ALS

2. If taking riluzole and/or edaravone, must be on a stable dose prior to Screening.

Key Exclusion Criteria:

1.History of a clinically significant non-ALS neurologic disorder

2.Inability to swallow capsules.

3.Human immunodeficiency virus (HIV) or current chronic/active infection with hepatitis C virus or hepatitis B virus

4.Women who are pregnant, planning to become pregnant, or are breastfeeding.

5.Use of non-invasive ventilation (NIV) or mechanical ventilation via tracheostomy, or on any form of oxygen supplementation.

6.Current or anticipated need of a diaphragm pacing system (DPS).

7.Currently using glucocorticoids or have a history of regular systemic glucocorticoid use within the last 12 months.

8.Previous exposure or treatment with glucocorticoid receptor modulators or antagonists.

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 50-300 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 various clinical symptoms of ALS according Revised Amyotrophic Lateral Sclerosis Functional Rating (ALSFRS-R) 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 days 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 ALS 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.