Dipyridamole in the Treatment of Uremic Restless Legs Syndrome: A Self-Controlled Study

Version Number: V 3.0
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Principal Investigator: Xia Yunfeng

Protocol Signature Page

I will, in accordance with Chinese GCP and the "Management Measures for Clinical Research Initiated by Investigators in Healthcare Institutions," diligently fulfill my responsibilities as an investigator, personally participate in or directly supervise this clinical research. I am familiar with the research protocol for this clinical study (Version Number: V 3.0; Version Date: July 7, 2025). I agree to perform the relevant duties in accordance with Chinese laws, the Declaration of Helsinki, Chinese GCP, and this research protocol. I will be responsible for making clinically relevant medical decisions, ensure that patients receive appropriate and timely treatment for any adverse events occurring during the study period, and record and report these adverse events as required by national regulations. I guarantee that the data will be recorded truthfully, accurately, completely, and in a timely manner. I will accept quality monitoring by the quality managers of the Clinical Research Management Department to ensure the quality of the clinical research. I commit to maintaining confidentiality regarding patient personal information and related matters. I agree to disclose expenses related to the clinical research upon request and agree to prohibit commercial and economic activities related to this study.

Principal Investigator Name: Xia Yunfeng
Principal Investigator (Signature): _____
Signature Date: _____ Year _____ Month _____ Day

I. Study Background:
Restless Legs Syndrome (RLS) is a common complication in uremic patients, significantly impacting their quality of life and prognosis. Many studies have found that RLS is closely related to nocturnal hypertension and the occurrence of cardiovascular events in uremic patients, affects patient sleep, leads to anxiety, depression, and decreased quality of life. The mortality rate of hemodialysis (HD) patients with RLS is significantly higher than that of patients without RLS [1]. Over the past few decades, dopaminergic drugs have been the mainstay of first-line treatment for RLS. Although these drugs show significant short-term efficacy, long-term use is characterized by short half-life, susceptibility to early morning rebound, and poor long-term efficacy [2, 3]. Recent studies suggest that nucleoside equilibrative transporter inhibitors, such as dipyridamole and ticagrelor, may control RLS symptoms by increasing endogenous adenosine-mediated tonic A1 receptor activation, potentially representing a new treatment strategy for RLS [4, 5]. Preliminary clinical studies have demonstrated that dipyridamole significantly improves patients' sleep quality and quality of life [6].
We will only screen patients who meet both the inclusion and exclusion criteria to participate in this study. Enrolled patients will receive dipyridamole free of charge, 50 mg each time, three times a day, for a total of 12 weeks. During the treatment period, we will regularly follow up on

the participants' condition. Participants will need to undergo assessments using the International Restless Legs Syndrome Study Group Rating Scale (IRLS), Clinical Global Impression scale (CGI), Medical Outcomes Study Sleep Scale (MOS-SS), etc., before and after treatment to evaluate the degree of symptom change. Through this treatment, we aim to understand the specific efficacy of dipyridamole in uremic patients with RLS, the extent to which the drug improves RLS sensory and motor symptoms and sleep conditions, observe the impact of dipyridamole on concomitant depression, anxiety, quality of life, etc., in maintenance dialysis patients, and also understand the efficacy and common adverse reactions of dipyridamole in clinical use in maintenance dialysis patients.

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[3] G P Y, Karen S, M L D, et al. A Narrative Review of the Lesser Known Medications for Treatment of Restless Legs Syndrome and Pathogenetic Implications for Their Use. [J]. Tremor and other hyperkinetic movements (New York, N.Y.), 2023, 13:7-7.
[4] Sergi F, César Q, William R, et al. Adenosine mechanisms and hypersensitive corticostriatal terminals in restless legs syndrome. Rationale for the use of inhibitors of adenosine transport. [J]. Advances in pharmacology (San Diego, Calif.), 2019, 84:3-19.
[5] Garcia-Borreguero D, Guitart X, Malo G C, et al. Treatment of restless legs syndrome/Willis-Ekbom disease with the non-selective ENT1/ENT2 inhibitor dipyridamole: testing the adenosine hypothesis[J]. Sleep Medicine, 2018, 45:94-97.
[6] Diego G, Celia G, José J G, et al. A Randomized, Placebo-Controlled Crossover Study with Dipyridamole for Restless Legs Syndrome. [J]. Movement disorders: official journal of the Movement Disorder Society, 2021, 36(10):2387-2392.

II. Study Objectives
To understand the efficacy and adverse reactions of dipyridamole in uremic patients with RLS, and its effects on concomitant depression, anxiety, sleep disorders, and quality of life in these patients.

III. Study Design and Methods:
1. Study Population: This is a single-center, self-controlled study. We plan to recruit 80 patients with uremia and RLS who are undergoing maintenance hemodialysis at the Blood Purification Center of the First Affiliated Hospital of Chongqing Medical University from July 2025 to December 2025.
Inclusion Criteria:
(1). Maintenance hemodialysis $\geqslant$ 3 months;
(2). Age between 18-75 years;
(3). Diagnosed with RLS according to the IRLSSG criteria and with an IRLS score > 15 points;
(4). Willing to cooperate with this study.
Exclusion Criteria:
(1). Comorbid with other cerebrovascular, muscular, or motor system diseases that affect the

assessment of RLS severity;
(2). Comorbid with active bleeding, such as gastrointestinal bleeding, cerebral hemorrhage, etc.;
(3). Patients already on long-term dipyridamole treatment;
(4). Use of other medications that may affect RLS severity within the past 3 weeks, such as dopamine receptor agonists/antagonists, tricyclic antidepressants, lithium, etc.;
(5). Patients with a history of psychiatric disorders;
(6). Pregnant or lactating women;
(7). Patients allergic to or intolerant of dipyridamole;
(8). Patients unable or unwilling to cooperate with this study.

2. Study Procedure:
(1) Screen patients undergoing maintenance hemodialysis at our hospital's dialysis center whose baseline International Restless Legs Syndrome Study Group Rating Scale (IRLS) score is >15 points.
(2) Patients who meet the inclusion criteria and are willing to sign the informed consent form.
(3) Collect general clinical data and pre-treatment laboratory parameters, including age, gender, body mass index, Charlson Comorbidity Index score, dialysis duration, serum iron, ferritin, serum parathyroid hormone, hemoglobin, albumin, and dialysis adequacy. Assess changes in patient condition before and after treatment using scales such as the International Restless Legs Syndrome Study Group Rating Scale (IRLS), Pittsburgh Sleep Quality Index (PSQI), Hamilton Depression Rating Scale (HAMD), Hamilton Anxiety Rating Scale (HAMA), Clinical Global Impression scale (CGI), Medical Outcomes Study Sleep Scale (MOS-SS), Epworth Sleepiness Scale (ESS), and KDQOL-SF™-36.
(4) All enrolled RLS patients will be instructed by designated personnel to orally take dipyridamole tablets, 50mg each time, three times a day, for a continuous period of 12 weeks.
(5) Collect information on the occurrence of drug-related adverse reactions during the study period, such as nausea, headache, dizziness, diarrhea, insomnia, fatigue, bleeding, etc. The follow-up period is 12 weeks.
(6) After the treatment concludes, collect the patients' general clinical data and laboratory parameters again.
(7) Statistical Analysis: Measurement data conforming to a normal distribution will be expressed as mean ± standard deviation ($\bar{x} \pm s$). Count data will be expressed as frequencies. Comparisons of means before and after treatment will be performed using paired-sample t-tests. Comparisons of rates before and after treatment will be performed using paired-sample chi-square tests. A P-value < 0.05 will be considered statistically significant. All statistical analyses will be performed using SPSS software.
(8) Evaluate changes in patient condition before and after treatment. Primary evaluation indicators: complete remission rate, partial remission rate, overall response rate, incidence of adverse events, dropout rate.

3. Sample Size Calculation:
Formula: $n = [(Z_{\alpha/2} + Z_{\beta}) * \sigma / \Delta]^2$
Where n is the sample size, $Z_{\alpha/2}$ is the two-sided quantile of the normal distribution corresponding to the significance level α (usually α=0.05, so $Z_{\alpha/2}$=1.96). Z_{β} is the quantile

of the normal distribution corresponding to the power (1- β) (power is usually set at 0.8, so Z β =0.84). σ is the standard deviation of the observation indicator, reflecting data dispersion. Δ is the expected mean difference before and after treatment, i.e., the minimum difference expected to be detected. Based on literature review and preliminary studies, we set σ =4 and Δ =2. Therefore, the calculated sample size n = 31.36. Rounding up, n=32. Considering potential dropouts, the sample size needs to be increased by 10%-20%, so the final sample size is set at 80.

4. Data Analysis and Statistical Methods:
Conduct statistical analysis on the obtained data: Measurement data conforming to a normal distribution will be expressed as mean ± standard deviation ($\bar{x}$ ± s). Count data will be expressed as frequencies. Comparisons of means before and after treatment will be performed using paired-sample t-tests. Comparisons of rates before and after treatment will be performed using chi-square tests. A P-value < 0.05 will be considered statistically significant.

Study Flowchart:
Patient Screening
↓
Collection of Patient Clinical and Laboratory Data
↓
Oral Administration of Dipyridamole Tablets under Guidance
↓ (12 weeks)
Collection of Drug-Related Adverse Reactions during the Study
↓
Post-Treatment Collection of General Clinical Data and Laboratory Parameters
↓
Statistical Analysis, Efficacy Evaluation, Manuscript Preparation

5. Safety Analysis and Content Evaluation:
Follow up and statistics on the types, number of cases, and severity grading of potential adverse reactions during dipyridamole treatment. Evaluate the causes of these adverse reactions and their causal relationship with the study drug. Calculate the incidence of adverse reactions. Main adverse reactions may include headache, dizziness, gastrointestinal discomfort (nausea, vomiting, diarrhea), hypotension, bleeding tendency, allergic reactions, etc.

6. Observation Indicators:
Primary Indicators: Restless Legs Syndrome symptom severity score (IRLS).
Secondary Indicators: Quality of life score (KDQOL-SF-36), Clinical Global Impression scale (CGI) score, Medical Outcomes Study Sleep Scale (MOS-SS) score, Pittsburgh Sleep Quality Index (PSQI) score, Epworth Sleepiness Scale (ESS) score, Hamilton Anxiety Rating Scale (HAMA) score, Hamilton Depression Rating Scale (HAMD) score, Self-Rating Anxiety Scale (SAS) score, Self-Rating Depression Scale (SDS) score.
Safety Indicators:
① Occurrence of adverse reactions: including but not limited to headache, dizziness, gastrointestinal discomfort (nausea, vomiting, abdominal pain, diarrhea), hypotension, bleeding

tendency (gingival bleeding, epistaxis, subcutaneous bruising, etc.), allergic reactions (rash, pruritus, dyspnea, etc.).

② Laboratory parameter monitoring:

* Hematological indicators: Regularly test complete blood count (including red blood cell count, hemoglobin, platelet count, etc.) and coagulation function (prothrombin time, activated partial thromboplastin time, fibrinogen, etc.) to monitor whether dipyridamole's antiplatelet aggregation effect increases bleeding risk or causes hematological abnormalities.

* Biochemical indicators: Test liver and kidney function parameters (e.g., ALT, AST, serum creatinine, blood urea nitrogen, etc.) to assess the drug's impact on liver and kidney function; monitor electrolytes (e.g., potassium, sodium, chloride, etc.) to observe if drug effects or changes in uremia condition lead to electrolyte disturbances.

③ Vital signs monitoring:

* Monitoring content: Regularly measure patients' blood pressure, heart rate, respiratory rate, body temperature, and other vital signs, with particular attention to blood pressure changes, alert for hypotension caused by the vasodilatory effect of dipyridamole.

IV. Study Risks and Benefits:

Study Risks: During dipyridamole treatment, patients may experience adverse events such as bleeding, including gastrointestinal bleeding, cerebral hemorrhage, subcutaneous bleeding, gingival bleeding, etc. If these occur, please inform the physician as soon as possible. The physician will take active intervention measures based on the specific situation to 尽量 ensure the protection of patient rights and interests. Furthermore, physicians will strictly maintain the confidentiality of patient medical records and information.

Study Benefits: The relevant examinations and collection of medical history data will contribute to a comprehensive understanding of the patient's disease and provide an optimal treatment plan. Under guidance, taking dipyridamole tablets may improve the patient's RLS sensory and motor symptoms as well as sleep quality.

Risk-Benefit Assessment: We predict that the benefits of this study significantly outweigh the associated risks.

V. Subject Recruitment

1. Recruitment Method: This study will recruit patients through the Dialysis Unit of the Department of Nephrology at the First Affiliated Hospital of Chongqing Medical University. The research team will screen patients who meet the inclusion criteria. All subjects are patients actively seeking medical consultation at the Dialysis Unit of the Department of Nephrology; no additional recruitment advertisements will be used.

2. Consideration Regarding Vulnerable Groups: This study strictly avoids recruiting vulnerable groups, including minors, pregnant women, prisoners, illiterate individuals, and individuals without capacity or with limited capacity. All subjects must be 18 years or older, have full capacity, be able to fully understand the study content, and provide signed informed consent.

VI. Informed Consent Process:

The informed consent process for this study will be strictly executed according to ethical norms, overseen by the project leader and research assistants to fully safeguard patient rights and the

right to informed consent.

(1) **Implementers of Recruitment and Informed Consent:** The recruitment and informed consent process for this study is primarily the joint responsibility of the project leader and research assistants. Research assistants are responsible for preliminary screening of patients against the inclusion and exclusion criteria and assisting the project leader in communicating study details to patients.

(2) **Specific Informed Consent Process:** After screening, the project leader or research assistant will conduct a face-to-face discussion with the patient, detailing the study's purpose, procedures, potential risks, and benefits, ensuring the patient fully understands the study. Provide the patient with a written informed consent form that is easy to understand, avoiding excessive technical terminology, ensuring the patient can read and comprehend it independently. Allow the patient ample opportunity to ask questions, and provide detailed answers to all queries, ensuring transparency and adequacy of the informed consent process.

(3) **Requirements for the Informed Consent Subject:** All subjects must be adults aged 18 or older with full capacity; no proxy signatures are required. The research team ensures voluntary participation and will absolutely not exert any form of pressure or inducement for patients to participate.

(4) **Avoiding Direct Recruitment by Treating Physician:** To avoid potential conflicts of interest or implicit pressure, the patient's treating physician will not participate in the informed consent process. Other members of the research team will communicate and explain the study content to the patient.

(5) **Voluntariness and Withdrawal Guarantee:** The informed consent form clearly states that participation is entirely voluntary, and the patient can unconditionally withdraw from the study at any stage, which will not affect their subsequent medical care. If a patient chooses to withdraw, the research team will provide necessary medical advice and ensure a smooth withdrawal process without additional burden.

(6) **Information Updates and Feedback:** If new information arises during the study that may affect patient rights or health, the project leader will promptly communicate with the patient and re-seek informed consent if necessary. Patients have the right to be informed about study-related information at any time, including research progress and data related to their own health status.

VII. Costs and Compensation

Funding for this project is provided by the 2025 Graduate Student Research Innovation Project of the First Affiliated Hospital of Chongqing Medical University. The required drug, dipyridamole tablets, will be provided free of charge by the research group. Corresponding liability insurance will be purchased for you. Provided you follow the research physician's instructions, if a drug-related adverse event attributable to the study drug or diagnostic tests and treatments required by the research protocol occurs and causes you harm, the physician will provide active treatment. The research group will bear the relevant medical costs and other expenses stipulated by law, including portions not covered by insurance. Treatment and examinations required for other concurrent diseases you may have are not within the scope of reimbursement. Additionally, we will provide free long-term follow-up of the patient's condition and offer long-term free treatment guidance, striving to improve RLS sensory and motor symptoms and sleep disorders. Since enrolled patients receive regular hemodialysis treatment at the Blood Purification Center of

the First Affiliated Hospital of Chongqing Medical University, and are already under regular treatment and laboratory follow-up there, our study only collects routine serological test results from the patients and will not impose additional economic burden. Furthermore, as our efficacy evaluation indicators are primarily reflected through scales, there is no corresponding compensation.

VIII. Data Acquisition and Quality Control

(1) **Data Acquisition Pathways:** Data sources include patient medical records, laboratory test results, and questionnaires and other clinical information collected during the research process. All data will be collected by the research team through standardized procedures and recorded in an encrypted electronic database.

(2) **Quality Control Measures:** A double-entry verification system will be used to ensure data entry accuracy. Regular checks for data completeness and consistency will be conducted. Any abnormal data will be promptly traced and corrected. The data management team is responsible for supervising the data collection and storage process to ensure compliance with ethical and regulatory requirements.

IX. Confidentiality of Subject Information

(1) **Data Usage Scope:** Subjects' personal data, including medical records and biological samples, will be used solely for the purposes of this research and will not be used for other purposes. All samples and data will be stored anonymously, identified only by a unique code, to prevent the disclosure of personal identity information.

(2) **Data Access Permissions:** Data access permissions are restricted to project team members, data analysts, and authorized reviewers from the ethics committee. When research results are published, they will be presented anonymously, without involving any personal information of the subjects.

(3) **Data Confidentiality Measures:** Data will be stored in encrypted databases meeting security standards, managed by designated personnel. Paper documents containing sensitive information will be stored in locked cabinets, inaccessible to unauthorized personnel.

X. Sharing of Results

After conclusion, all relevant results will be published in scientific journals and may be presented at academic conferences. Research findings will be shared with the global scientific community to promote academic exchange and knowledge updates in this field.