Efficacy of Rapamycin (Sirolimus) in the Treatment of BRBNS, Hereditary or Sporadic Venous Malformation

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I. Background

Blue rubber bleb nevus syndrome (BRBNS) is a rare systemic syndrome featuring venous malformation and hemangiomas. Affected organs include skin and gastrointestinal tract. Patients manifest venous malformation (VM) at birth. Main histologic features of VM are dilated and distorted veins surrounded by sparse and irregular smooth muscle cells. The size and number of VM lesions in BRBNS increase gradually with age. Patents' skin develops 1-2cm of blue-violet, compressible, and hyperkeratotic VM lesions, mostly in palms and soles. Lesions cause local deformity, pain and intravascular coagulopathy. The latest menifests elevated D-dimers and may lead to disseminated intravascular coagulation (DIC). Risk of DIC is even higher under surgical treatment. Lesions of BRBNS can be found in the mucosa of the whole gastrointestinal tract. Small intestine is the most commonly affected organ. Lesions can cause gastrointestinal bleeding, intussusception, intestinal torsion and even intestinal necrosis[1]. Due to long-term chronic gastrointestinal bleeding, many patients need to take life-long iron supplementary treatment and receive blood transfusions repeatedly. VM lesions of BRBNS can also affect organs in pelvic, abdominal and thoracic cavity. BRBNS is such a devastating disease, traditional drugs such as glucocorticoids, interferon-α, propranolol and intravenous iron are not effective. Besides surgical resection of affected tissue and repeated sclerotherapy, there is no validated targeted drug treatment.

Sporadic BRBNS cases have been reported. There are also a few familial cases consistent with autosomal dominant inheritance pattern[2-6]. Soblet et al[1] show that BRBNS is caused by somatic mutation of TEK gene, which encodes a receptor of angiogenin, endothelial tyrosine kinase receptor TIE2. Multifocal VM lesions of BRBNS patients are caused by double (*cis*) mutations, i.e., two somatic mutations on the same allele of the gene. Germline mutations of TEK gene can cause venous malformation, cutaneous and mucosal (VMCM), a rare autosomal dominant hereditary disease. Several repetitive mutation sites of TEK gene have been observed in sporadic BRBNS patients[1]. These mutations result in ligand-independent activation of TIE2 and PI3K/AKT signaling pathways, which in turn promote cell survival, migration, invasion and colonization.

In addition to multifocal VM lesions, Soblet et al. also foumd that about 20% of unifocal VM patients carry PIK3CA gene (encoding Class I p110α catalytic subunit of PI3K) mutation, which also activates the PI3K/AKT signaling pathway. Clinically, venous malformation diseases caused by TIE2 and PIK3CA somatic mutations are almost indistinguishable, suggesting that the PI3K/AKT signaling pathway plays a key role in BRBNS[1].

An important protein kinase mTOR (mammalian target of rapamycin) downstream of the PI3K/AKT pathway can serve as a potential therapeutic target for the venous malformation syndromes mentioned above. Experiments on mice have shown that mTOR inhibitor rapamycin can control the progression of VM lesions. It can also relieve VM lesions caused by PIK3CA and TEK mutations in humans[7]. There are a few cases reported using rapamycin (sirolimus) as treatment, and achieving satisfactory effects without significant adverse events[8-11]. Large-scale prospective studies are needed to demonstrate the efficacy of rapamycin for BRBNS.

II. Objective

1. To evaluate the safety of sirolimus in treatment of blue rubber blisters syndrome (BRBNS), hereditary cutaneous and mucosal venous malformation (VMCM), and sporadic multiple VM.
2. To evaluate the efficacy of sirolimus in treatment of blue rubber blisters syndrome (BRBNS), hereditary cutaneous and mucosal venous malformation (VMCM), and sporadic multiple VM.

III. Research Design

1. This study is a prospective, nonrandomized, open-label, single-arm clinical trial. The sirolimus capsule (Rapamycin, Yixinke®), 0.5 mg/grain*20/box, is produced by North China Pharmaceutical Co., Ltd.
2. All patients who meet inclusion criteria and sign informed consents receive sirolimus treatment for at least 6 months. Patients are followed up in a regular schedule.
3. No less than 20 patients should be enrolled.

IV. Selection of subjects

A. Inclusion criteria

(1) Patients diagnosed with BRBNS, VMCM, sporadic multiple VM, or large single VM;
(2) Age and gender are not limited;
(3) Physical status ECOG 0~3;
(4) Organ function is good, biochemical examination meets the following conditions:
a) AST ≤ 2.5 × upper limit of normal value (ULN),
b) ALT ≤ 2.5 × upper limit of normal value (ULN),
c) serum total bilirubin ≤ 1.5 × upper limit of normal value (ULN),
d) creatinine ≤ 1.5 × upper limit of normal (ULN);
(5) Patients volunteer to participate in the trial. Participant or his/her legal guardian signs the informed consent form.

B. Exclusion criteria

(1) Patients need emergency surgery due to intestinal obstruction, intussusception, or gastrointestinal bleeding;
(2) History of surgery within 1 month;
(3) Allergic to rapamycin;
(4) Any disease or condition that may affect the study implementation or result interpretation, including:
a) known hemoglobinopathy
b) concomitant gastrointestinal infections
c) severe heart, liver, kidney and other serious concomitant diseases that may endanger lives
(5) Pregnant or lactating women;
(6) Alcohol or drugs (eg, laxatives) abusers;
(7) Participate in another clinical trial that may affect his or her study within one month;
(8) The investigator believes to be not suitable for other reasons.

C. Exit criteria

(1) An allergic reaction occurs.

(2) The patient requests withdrawal: at his or her own discretion or at the request of his or her legal representative. Subjects may refuse to participate in further studies at any time without providing reasons. Subjects will not be affected because of such decision.

(3) Subjects are required to withdraw from the study in certain special circumstances (eg, there is significant issues of compliance, safety, or surgery for venous malformations)

(4) Other situations in which the study must be terminated. For example, the investigators believe that continuing the study may be harmful to the health of subjects.

D. Rejection criteria

(1) Patients who violate the requirements of the test protocol

(2) Patients with poor-recording, incomplete, or inaccurate data

V. Therapeutic schedule

A. Enrollment

Before starting the study, the investigator clearly and colloquially explains the study and its potential risks and benefits to the patient or his/her authorizer. Patient or his/her authorized and the investigator sign and date the informed consent form. Only after signing the informed consent form can patient or his/her authorizer enter the screening section. Patients who sign a written informed consent need to be assessed to determine if the patient meets the study criteria:

1) Demographic data and medical history of the patient;

2) vital signs;

B. Children's dosage regimen

Oral administration

The initial dose of oral rapamycin intake is 1 mg/m^2 of body surface area. Drug trough concentration in patient's blood is maintained at 5-10 ng/ml.

C. Adults' dosage regimen

Oral administration

1) Initial dose and dosing schedule: sirolimus 2mg, once a day. Maintain drug trough concentration of 5-10ng / mL in blood. If adverse events are too severe to tolerate, daily dosage can be reduced by 0.5mg every week. The minimum dosage is 1 mg/day. If patient still cannot tolerate minimum dosage, withdraw from the study. If patient could tolerate the possible adverse events, continue to take the original dose for 3 months, and evaluate improvement of disease condition.

2) Subsequent dose and dosing schedule: After taking sirolimus 2 mg once a day for 3 months, those with improved condition continue to take the original dose. If improvement of disease condition is not obvious, change dosage to 2.5mg-3mg once a day. If this dosage cannot be tolerated, patients may withdraw from the study. If it can be tolerated, continue to take for another 3 months and reassess the improvement of condition.

3) Blood drug concentration test: Blood samples are taken at 1st, 3rd and 6th months of dosage schedule.

4) Iron supplementation: For subjects with hemoglobin ≤ 10.9 g / dl, iron supplementation should be provided according to the standard dosing regimen, but iron supplementation is

not considered as study treatment and will be recorded as concomitant medication.

VI. Follow-up plan and content

A. Evaluation before the trial

Each subject should complete the following items in four weeks before the treatment:

1) Complete medical history
2) Detailed personal profile
3) VAS pain score
4) Quality of Life (Qol) Rating Scale
5) Frequency of blood transfusion therapy
6) A physical examination
7) Blood and urine routine examination
8) Liver and kidney function, blood lipid test
9) Coagulation I+D dimer examination
10) abdominal and pelvic cavity contrast-enhanced CT + small intestine reconstruction CT examination (for patients with gastrointestinal system affected)
11) Digestive system endoscopy (for patients with gastrointestinal system affected)
12) Ultrasound, MRI examination of the lesion
13) (Female of childbearing age) blood/urine pregnancy test

B. Evaluation during sirolimus treatment

Adverse reactions are evaluated weekly during the first month of drug treatment. The efficacy and safety are then evaluated at the 3rd and 6th months, and the content of the examination is the same as the pre-trial evaluation.

1) Efficacy assessment:
   a. Clinical symptom assessment (including VAS pain score, gastrointestinal bleeding, quality of life (Qol) evaluation, frequency of blood transfusion treatment and other related symptoms);
   b. Changes in hemoglobin;
   c. Coagulation I+D-dimer examination;
   d. Nutritional status (including albumin, etc.);
   e. Ultrasound, MRI examination of the lesion, abdominal and pelvic cavity contrast-enhanced CT + small intestine reconstruction CT examination, digestive system endoscopy to measure the size, quantity and total load of VM lesions;
2) Safety assessment: Safety parameters (such as asking adverse events, measuring laboratory values, vital signs, gastrointestinal bleeding, etc.) will be closely monitored on a regular basis throughout the study.
3) Treatment compliance: In order to monitor compliance, subjects are required to complete an electronic diary during the entire study period. Dates of drug intake will be tracked by this diary. At every visit, the investigator and the subject will review the electronic diary data together.

C. Evaluation after treatment

Evaluation of curative effects and safety is conducted at the end of the drug therapy, six and twelve months after the treatment. Its content is the same as pre-trial evaluation.

VII. Records and reports of adverse events

A. Adverse events

The term of adverse events encompass any signs, symptoms, syndromes or diseases affecting health condition of subjects during the trial. The term also includes clinically relevant conditions found by laboratory test or other diagnostic procedures. It may call for unplanned treatment, or lead to withdrawal from the trial. Adverse events may be: new disease; deterioration of symptoms accompanying disease progression during treatment; certain events not related to the trial; combination of one or more events above.

Serious adverse events

At any dose of the test drug or at any time during the observation period, the following adverse events occurs: death; immediate life-threatening events; requirement of hospitalization or prolonged hospital stay; disability; congenital malformations; other events with important medical implications (those do not immediately endanger life or cause death or require hospitalization, but may harm the patient or require interventions to prevent one of the consequences defined above) and requiring medical treatment to prevent permanent injury.

B. Adverse reactions

Harmful and unexpected reactions caused by pharmaceutical applications under normal use of drugs and prescribed doses.

C. Degree of adverse reactions:

1) Mild: causing mild discomfort which can be tolerated and does not affect daily life; under this circumstance, the subject does not need to discontinue medication.
2) Moderate: causing discomfort which affects patients' daily life.
3) Severe: affecting daily life; cannot be endured by the subject or cause organ damage and need to withdraw medication.
4) Very serious: risk of disability or death; require emergent rescue and drug withdraw.

D. Evaluation of the relationship between adverse reactions and experimental drug

According to the 5-level judgment system, the relationships can be divided into 5 types: definitely related, very likely related, may be related, may be irrelevant, and definitely irrelevant. The first three are counted as adverse reactions, in which case the incidence of adverse reactions and adverse events should be reported.

D. Record and report

The investigator should explain to the patient or his/her legal guardian that it is required to truthfully reflect the changes in the condition after administration. Doctors should avoid suggestive questions. While observing the efficacy, pay close attention to the observation of adverse events or unanticipated side effects (including symptoms, signs, laboratory tests), analyze the causes, make judgments, and report the incidence of adverse reactions and adverse events.

For adverse events occurring during the trial, time, symptoms, extent, duration, treatment measures, outcomes, and some other items should be recorded in the case report form to evaluate the correlation with the test drug. Investigator should record it in detail, and then signs and dates.

E. Treatment of adverse events

1) reporting system

Any adverse events, such as subjective discomfort and abnormal laboratory tests, should be taken seriously and carefully analyzed. Immediate measures should be taken to protect safety of the subject.

2) treatment procedure

Physicians are pre-trained to prevent certain types of adverse events or reactions that may occur in the study, so as to protect safety of patients. Treatment procedure of adverse event should be recorded in detail. Persistence and disappearance of adverse events should also be recorded.

3) treatment of serious adverse events

On the occurrence of any serious adverse event, research physician should report to primary PI within 24 hours, in addition to emergency treatment.

4) follow-up of unresolved adverse events

All adverse events should be tracked until they are properly resolved. Adverse events with abnormal laboratory values should be tracked until the value returns to baseline.

VIII. Statistical analysis

Statistical analysis will be conducted by SPSS.

A. Methods of statistical analysis

The categorical variables will be described by frequency distribution (number of cases and percentage). Continuous variables will be described with mean, median, minimum, maximum, first and third quartile. The confidence interval of all parameters is set to be 95%. All analyses will be conducted with SPSS.

B. Contents of statistical analysis

1) General data analysis: Demographic variables and baseline characteristics will be summarized comprehensively by mean of descriptive statistics and/or appropriate frequency tables.

2) Evaluation of efficacy: According to follow-up and reexamination results, the patients' quality of life, size and volume of the lesions, tumor load control and improvement of symptoms will be analyzed.

3) Analysis of adverse events: Side effects and adverse events during medication will be assessed.

4) Evaluation of the relationship between adverse events and the tested drug

IX. Measures of quality control

1. Supervision by the project leader
2. Establish a standardized evaluation method, unify various diagnostic criteria and efficacy criteria
3. Formulate plans for monitoring and treatment of adverse reactions

4. All researchers are trained before the study begun
5. Designate quality controllers and develop a plan for quality control, with regular inspections
6. Devise a follow-up plan and system

X. Expected research results

The results will be published in a paper and fed back to the subjects.

XI. Potential risks, benefits or advantages of this study

As a drug that has been on the market for years, it is applied to this study beyond its indication. It is recommended by doctors considering that its benefits outweigh its risks and the subjects have no better choice. For subjects, it may relieve their symptoms and improve quality of life, and complications associated with surgery and damage to intestinal function can be avoided. The testing drug is free during the study and subjects should bear the risks of treatment.

XII. Data preservation and confidentiality

It will be stated in the informed consent that personal information of subjects will be strictly confidential in this study. Results of this study may be published in scientific paper, but personal information of subjects, including their names, will not appear in the paper. All data relevant to this study will be preserved by Peking Union Medical College Hospital and Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, and strict confidentiality measures will be established.

XIII. Measures to protect subjects and minimize risks

In the whole course of this study, safety supervision will be carried out by experts from the digestive surgery department of our hospital to strictly control diagnostic criteria and treatment indicators of BRBNS, VMCM and VM, making the collected clinical data meet international diagnostic criteria and providing favorable treatment for these patients.