Study Protocol

TADAFER II:

A multicenter phase II trial of the efficacy and safety of tadalafil in fetus with early-onset growth restriction.

Trial registration: UMIN Clinical Trials Registry UMIN000023778.

Version 1

Date 25-August-2016

Contents

SYNOPSIS

1. Objectives

This multicenter randomized controlled phase II trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in fetus with fetal growth restriction (FGR).

2. Study eligibility

This study will include fetuses and their mothers who meet the following conditions:

(1) Pregnant women ≥ 20 years.

(2) Estimated fetal weight (EFW) less than 1.5 standard deviations of the mean EFW for gestational age.

(3) Gestational age between 20 + 0 and 33 + 6 weeks.

(4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014).[1]

(5) Singleton pregnancy.

(6) Signed written informed consent.

3. Treatment

Fetuses with FGR will be randomized to receive either the conventional management of FGR according to the guidelines for obstetrical practice in Japan[1] or once-daily treatment with 20 mg tadalafil added to the conventional management until delivery.

4. Target sample size and duration of the study

Duration of the study: date of ethics approval to February 2021.

Target sample size: 140 singleton fetuses and their mothers.

5. Endpoints

(1) Primary endpoint: fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).

(2) Secondary endpoints

1) Completion rate of the treatment regimen

2) Efficacy endpoints: estimated fetal weight (g), fetal growth velocity in the two weeks after the protocol-defined treatment (g/day), fetal growth velocity in the two weeks after one week of

the protocol-defined treatment (g/day), fetal growth rate (%/day), fetal head circumference (cm), Doppler imaging of umbilical arterial blood flow, deepest amniotic fluid pocket (cm), prolongation of gestational age at birth (days), birth weight (g), gestational age (GA) at birth, Apgar score, umbilical artery pH and base excess values, incidence rate of pre-eclampsia, and pediatric developmental assessment until 1.5 years of age.

3) Safety endpoints: incidence rate of obstetric complications, perinatal mortality, and neonatal mortality.

6. Secretariats

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1. VOLUNTARY PARTICIPATION AND WITHDRAWAL

Participation in this study is on a voluntary basis. Refusal to participate will incur no penalty or loss of benefits to which patients are otherwise entitled to. The subject may withdraw at any time without penalty.

2. BACKGROUD AND OBJECTIVES

Neonatal intensive care has improved over the past few decades, and morbidity among infants, including those who are premature, continues to decline. Premature infants with intrauterine growth restriction, however, still have high mortality and morbidity. The multicenter survey[2] of very low birth weight (VLBW) infants in Japan conducted by Kusuda and Ikeda *et al.* revealed that mortality in neonatal intensive care units (NICU), of small gestational age (SGA) infants born before 30 weeks gestation, was significantly higher than that of appropriate for gestational age (AGA) infants (unpublished data). To prevent fetal growth restriction (FGR), nutritional and dietary supplementation, bed rest, and aspirin therapy have been investigated, but there is insufficient evidence for the routine indication of any of these treatments.[3] There is also no proven therapy to reverse or ameliorate established FGR.[4]

Increases in uteroplacental blood flow during pregnancy via angiogenesis and vasodilation contribute to adequate fetal growth. Vasodilation in the uteroplacental unit is considered to be due to the production and local release of nitric oxide (NO), which stimulates cyclic guanosine monophosphate (cGMP) production.[5] cGMP is inactivated mainly by phosphodiesterases (PDE), and the predominant PDE isoform present in the vascular smooth muscle is PDE5. Because inhibitors of PDE5, which is a cGMP-specific PDE, exert their pharmacological action by dilating arteries and increasing blood flow, as proven in erectile dysfunction and pulmonary hypertension, recent studies have suggested a potential therapeutic role for PDE5 inhibitors in treating FGR.[6] Sildenafil, a selective PDE5 inhibitor, has been shown to improve endothelial function in myometrial small arteries removed from women with pre-eclampsia and FGR.[7,8] However, although sildenafil has been reported to affect maternal hypertension, it has not been shown to affect FGR in studies in FGR model rats induced by L-NG-nitroarginine methyl ester (L-NAME) treatment. In these studies, FGR was not affected by sildenafil except in one report, by Baijnath *et al.*[9,10,11,12] Baijnath *et al.* demonstrated that L-NAME-induced FGR was improved by sildenafil treatment from 4 days postcoitum (d.p.c.) to

8 d.p.c. but not from 8 d.p.c. to 14 d.p.c.[11] Chorioallantoic attachment occurs at 8 d.p.c., and the mature circulatory pattern of maternal blood through the placenta is established by 10 d.p.c. in mouse placenta.[13,14] In considering the development of fetoplacental circulation in rodents, the effect of sildenafil on fetal growth associated with placental blood flow via an NO-dependent pathway was not manifested. In a clinical study, it was reported that sildenafil was associated with increased fetal abdominal circumference (AC) growth velocity in severe early-onset FGR, but the authors did not report on fetal growth velocity and birth weight.[15] Recently, the STRIDER UK group has found no evidence of a beneficial effect of sildenafil on survival or short-term neonatal outcomes.[16]

Tadalafil is also a selective PDE5 inhibitor and has a longer half-life and a more rapid onset of action than sildenafil.[6] Tadalafil has been used to treat pulmonary hypertension in pregnant women and the Food and Drug Administration in the United States has rated tadalafil as pregnancy category B.[17] When taking sildenafil with a high-fat meal, the time to maximum plasma concentration increases and the peak plasma concentration falls.[18] In contrast, Forgue *et al.* reported that food intake had a negligible effect on the bioavailability of tadalafil, and also reported that there was no clinically meaningful effect of gender on tadalafil pharmacokinetics.[19] Our animal experiments demonstrated that tadalafil treatment dilates the maternal blood sinuses in the placenta, which leads to increased placental growth factor (PlGF) production, and contributes to facilitating fetal growth.[20] Because tadalafil treatment was started after blood spaces in the placenta were narrowed by L-NAME treatment and elevated urinary excretion of cGMP in these animal experiments, we can safely presume that tadalafil treatment contributes to facilitating fetal growth in the context of the mechanisms associated with NO signaling. In addition, we retrospectively analyzed 11 Japanese singleton pregnant women with FGR who received tadalafil along with conventional management for FGR at Mie University Hospital from July 2015 to February 2016 (tadalafil group).[21] These women were matched for maternal age, parity, gestational age (GA), and estimated fetal weight at enrollment with 14 singleton pregnant women who had received only the conventional management for FGR in 2014 (conventional management group). The conventional management for FGR was performed according to the guidelines for obstetric practice in Japan.[1] This retrospective study showed that both fetal growth velocity from enrollment to birth and birth weight were significantly higher in the tadalafil group than in the conventional management group. Moreover, the prevalence of respiratory distress syndrome (RDS) was significantly lower in the

tadalafil group than in the conventional management group. After the retrospective study, we conducted a phase I clinical trial to ensure the safety of tadalafil treatment for FGR.[22] There were no serious maternal adverse events for daily tadalafil doses of 10 mg, 20 mg, and 40 mg. More patients who were administered 40 mg tadalafil daily experienced mild adverse events than those administered 10 mg or 20 mg tadalafil daily. In regards to fetal adverse events, intrauterine fetal death occurred in one case. In this case, the pregnant woman was prescribed 40 mg tadalafil daily and fetal growth had been progressing at a rate of 22 g/day. At 36 weeks gestation, fetal movement suddenly ceased and a diagnosis of intrauterine fetal death was made. Thereafter, the fetus was delivered vaginally, and velamentous insertion of the umbilical cord was identified. Immediately, the safety evaluation committee investigated the incident's relationship to tadalafil. This committee analyzed the case and concluded that the intrauterine fetal death was due to velamentous insertion of the umbilical cord.[23] We concluded that tadalafil treatment was feasible in pregnant women with FGR.[22]

Based on the above, we have hypothesized that tadalafil therapy will safely increase the likelihood of increased fetal growth in fetuses with FGR and have designed this multicenter randomized controlled phase II study to establish evidence for fetal therapy with tadalafil. This study, funded by the Japan Agency for Medical Research and Development (AMED), will prospectively evaluate the safety and efficacy of tadalafil in FGR with the participation of major medical centers providing treatment for fetuses with FGR according to the guidelines for obstetrical practice in Japan. Fetuses will be randomized to receive either the conventional management for FGR, according to the guidelines in Japan, or a once-daily treatment with 20 mg of tadalafil along with the conventional management, until delivery. Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day) has been defined as the primary endpoint in this study. To minimize bias in terms of fetal baseline condition and timing of delivery, a fetal indication for delivery is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database, in which the 82 level III perinatal centers were registered.[2] The investigator will evaluate fetal baseline conditions at enrollment and will decide the timing of delivery based on this fetal indication. For other complications such as preterm labor, rupture of the membranes, and hypertensive disorder of pregnancy, the investigator will follow guidelines for obstetric practice in Japan.[1] The investigator will enter the patients' data into the Case Report Form on the website of this clinical trial (the Clinical Trial Data Management System). Infants will be followed up and evaluated for physiological and neurological development until 1.5 years of age.

3. RESEARCH ORGANIZATION AND PARTICIPATING INSTITUTIONS

(1) Corresponding	Mie University	Tomoaki Ikeda (Principal Investigator)
(2) Collaborator	Showa University	Akihiko Sekizawa
	Osaka University	Tadashi Kimura
	Nagoya University	Tomomi Kotani
	Mie Chuo Medical Center	Yuka Maekawa
	Municipal Yokkaichi hospital	Kenji Nagao
	Ise Red Cross Hospital	Tomohisa Kihira
	St. Marianna University	Nao Suzuki
	Juntendo University	Satoru Takeda
	The Jikei University	Aikou Okamoto
	Toho University	Masahiko Nakata
	Yokohama City University Medical Center	Shigeru Aoki
	Kanagawa Children's Medical Center	Hiroshi Ishikawa
	Ehime University	Takashi Sugiyama
	Hamamatsu University School of Medicine	Naohiro Kanayama
	Osaka Medical College	Masahide Ohmichi
	Niigata University	Takayuki Enomoto
	Showa University Northern Yokohama Hospital	Kiyotake Ichizuka
	Showa University Koto Toyosu Hospital	Katsufumi Otsuki
	Gifu University	Kenichiro Morishige
	University of the Ryukyu	Yoichi Aoki
	Shiga University	Takashi Murakami
	Shinshu University	Tanri Shiozawa
	Ehime Prefectural Central Hospital	Hiroshi Ochi
	Akita University	Yukihiro Terada
	Tokyo Metropolitan Bokutoh Hospital	Hironobu Hyodo
	Kyorin University	Mitsutoshi Iwashita
	Tokyo Metropolitan Tama Medical Center	Akira Kohyama
	Kuwana East Medical Center	Yoshihito Sasaki
	Kanazawa University	Hiroshi Fujiwara
	Nagasaki Medical Center	Ichiro Yasuhi

University of Toyama	Shigeru Saito
Yamaguchi University	Norihiro Sugino
Toyota Memorial Hospital	Hidenori Oguchi
Kainan Hospital	Tadashi Sumi
Dokkyo Medical University	Susumu Miyashita
Saga Hospital	Makoto Nomiyama
Kyoto Prefectural University	Jo Kitawaki
Toyama Central Prefectural Hospital	Hiroshi Funamoto
Sapporo City General Hospital	Kazuhiko Okuyama
Kagoshima University	Hiroaki Kobayashi
Mie Prefectural General Medical Center	Hirohiko Tanaka
Kyoto University	Masaki Mandai
Sakakibara Heart Institute	Shinji Katsuragi
University of Fukui	Yoshio Yoshida

(3) Safety Evaluation Committee

The Safety Evaluation Committee is independent from research organization, and responsible for the overall safety of this clinical study. To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will review adverse events of tadalafil. The Safety Evaluation Committee consists of Dr. Makoto Maeda (Board Certified Member of the Japan Society of Obstetrics and Gynecology) and Dr. Yoshiaki Miyake (Board Certified Member of the Japan Society of Obstetrics and Gynecology).

(4) Protocol Evaluation Committee

The Protocol Evaluation Committee is an organization of the execution of this study. All experimental protocols are evaluated and approved by the Protocol Evaluation Committee.

(5) Data Coordinating Center at the Clinical Research Support Center in Mie University Hospital

This center supports the data management, and statistical analysis and reporting of the study. This consists of Dr. Masakatsu Nishikawa (chairperson), Ms. Yuki Nishimura (data manager), and Dr. Toru Ogura (statistics).

(6) Secretariats

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4. STUDY SUBJECTS AND METHODS

(1) Study Sites and Subjects

1) Study Sites

This is a multicenter randomized controlled phase II trial, in which the Clinical Research Support Center in Mie University Hospital serves as the data center. Since this trial has been designed to prospectively evaluate the efficacy and safety of tadalafil treatment in FGR, fetuses will be randomized to receive either the conventional management for FGR, according to the guidelines in Japan[1] or a once-daily treatment with 20 mg of tadalafil along with the conventional management at the data center. Case registration requires the approval of the Ethics Committee. The following institutions will participate in this clinical trial:

Mie University, Showa University, Osaka University, Nagoya University, Mie Chuo Medical Center, Municipal Yokkaichi hospital, Ise Red Cross Hospital, St. Marianna University, Juntendo University, the Jikei University, Toho University, Yokohama City University Medical Center, Kanagawa Children's Medical Center, Ehime University, Hamamatsu University School of Medicine, Osaka Medical College, Niigata University, Showa University Northern Yokohama Hospital, Showa University Koto Toyosu Hospital, Gifu University, University of the Ryukyu, Shiga University, Shinshu University, Ehime Prefectural Central Hospital, Akita University, Tokyo Metropolitan Bokutoh Hospital, Kyorin University, Tokyo Metropolitan Tama Medical Center, Kuwana East Medical Center, Kanazawa University, Nagasaki Medical Center, University of Toyama, Yamaguchi University, Toyota Memorial Hospital, Kainan Hospital, Dokkyo Medical University, Saga Hospital, Kyoto Prefectural University, Toyama Central Prefectural Hospital, Sapporo City General Hospital, Kagoshima University, Mie Prefectural General Medical Center, Kyoto University, Sakakibara Heart Institute, and University of Fukui.

2) Subjects and Diagnostic Methods

All patients have to meet all inclusion criteria without violating any of the exclusion criteria listed below. All subjects will be followed-up until the end of the study.

Inclusion Criteria

(1) Pregnant women ≥ 20 years.

(2) EFW less than 1.5 standard deviations of the mean EFW for GA.

(3) GA between 20 + 0 and 33 + 6 weeks.

(4) The expected date of confinement is determined using the criteria of the guidelines for obstetrical practice in Japan (2014).

(5) Singleton pregnancy.

(6) Signed written informed consent.

Exclusion Criteria

(1) A result from the antepartum fetal tests, done at enrollment, which indicates that delivery should be attempted.

(2) A history of allergy to tadalafil.

(3) Concurrent medications that interact adversely with tadalafil.

(4) Contraindication of tadalafil treatment due to renal disease.

(5) Contraindication of tadalafil treatment due to liver disease.

(6) Contraindication of tadalafil treatment due to uncontrolled arrhythmia, hypertension (BP >170/100 mmHg), and hypotension (BP <80/40 mmHg).

(7) Fetus with suspected chromosomal disorder and/or multiple congenital anomalies.

(8) Contraindication of tadalafil treatment due to retinitis pigmentosa, coagulation defect, active gastric and/or intestinal ulcer, and venous obstructive disease.

(9) The investigator decides to entry inappropriate.

Rationale for Eligibility Criteria

- When diagnosed as FGR, the mean EFW for GA but not the mean birthweight for GA should be used, and the estimated date of confinement using fetal measurements obtained during the early stage of pregnancy should be confirmed according to the guidelines for obstetrical practice in Japan (2014) in Inclusion Criteria Nos. 2 and 4.[1]
- The lower age limit (20 weeks gestation) of Inclusion Criterion No. 3 is determined referring to the previous study protocol about the treatment for FGR.[24] The upper limit of <34 weeks gestation is based on infant survival rate in the NICU categorized by birth weight and gestational week at birth from the Japanese neonatal research network database (http://nponrn.umin.jp/index.html Japanese-only website), in which indicates that treatments are prioritized over elective delivery (Figure 1).

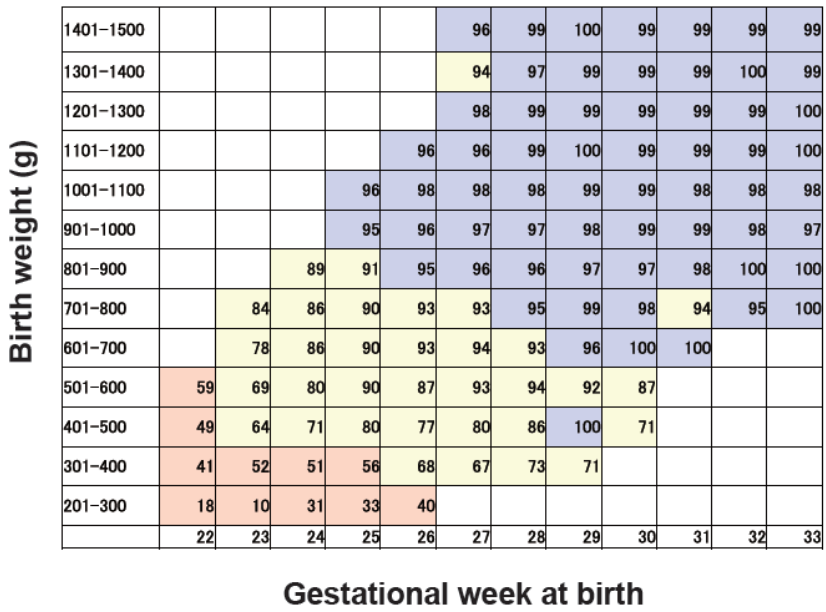

Figure 1. Infant survival rate in NICU assembled by birth weight and gestational week at birth (%).
This figure is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database. The survey data included infant survival rates in the NICU, categorized by birth weight and gestational week at birth.2 The infant survival rate data acquired from the survey were preprocessed with the moving average method and divided into three groups. The first group was defined as "Zone 1" where the infant survival rate in the NICU was less than 60% (highlighted by a red background). The second group was defined as "Zone 2" where the infant survival rate in the NICU ranged from 60 to 95% (highlighted by a yellow background). The third group was defined as "Zone 3" where the infant survival rate in the NICU was 95% or higher (highlighted by a blue background).

• Only singletons will be included in this study (Inclusion Criterion No. 5) to accurately evaluate clinical improvements, because fetal growth in multifetal pregnancies is different from that of singleton pregnancies.[23]

• The informed consent of the mother provides the ethical basis of this study (Inclusion Criterion No. 6).

• To minimize bias in terms of fetal baseline condition at enrollment, a fetal indication for delivery is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database, in which the 82 level III perinatal centers were registered. The survey data included infant survival rate in the NICU, categorized by birth weight and gestational week at birth (Figure 1).[2] The infant survival rate data acquired from the survey were preprocessed with the moving average method and divided into three groups. The first group was defined as "Zone 1" where the infant survival rate in the NICU was less than 60%. The second group was defined as "Zone 2" where the infant survival rate in the NICU ranged

from 60 to 95%. The third group was defined as “Zone 3” where the infant survival rate in the NICU was 95% or higher. All patients in our study will undergo antepartum fetal tests consisting of the evaluation of fetal well-being by ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA, to evaluate possible pregnancy termination by the investigator at enrollment (Table 1. Exclusion Criterion No. 1).

Table 1. A fetal indication for delivery in the TADAFER II study.[1,23,25]

Infant survival rate in the NICU (See Figure 1)	
Zone 1	Decide timing of delivery depending on available therapeutic measures at NICU in each institute.
Zone 2	Consider delivery if at least one of three findings is made, but place give high priority on the determination by the investigators. 1. Reversed umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.[25]
Zone 3	Consider delivery if at least one of five findings is made, but place give high priority on the determination by the investigators. 1. Reversed or absent umbilical artery blood flow during diastole. 2. Score less than 4 on the fetal biophysical profile score. (Score less than 6 on the fetal biophysical profile score if oligohydramnios is present.) 3. Feat heart rate patterns in the orange or red category for more than 30 minutes.[25] 4. Positive contraction stress test. 5. Impaired fetal head circumference growth for more than 2 weeks.

- Patients who have contraindications for tadalafil treatment will be excluded (Exclusion Criteria from No.2 to No.7).
- Regarding exclusion criteria No.9, this study excludes mothers with mental or psychiatric problems, since poor judgment capabilities that are often associated with such conditions may not be compatible with Inclusion Criterion No. 6.

(2) Study Design

This study is a multicenter randomized controlled phase II trial.

(3) Methods

In this multicenter clinical study, each study site will obtain ethics approval of the protocol before its implementation.

Registration

This study protocol defines all the procedures and schedules that the investigator must abide by to complete this clinical study, including patient selection and registration, fetal treatment of FGR, and follow-up (Figure 2).

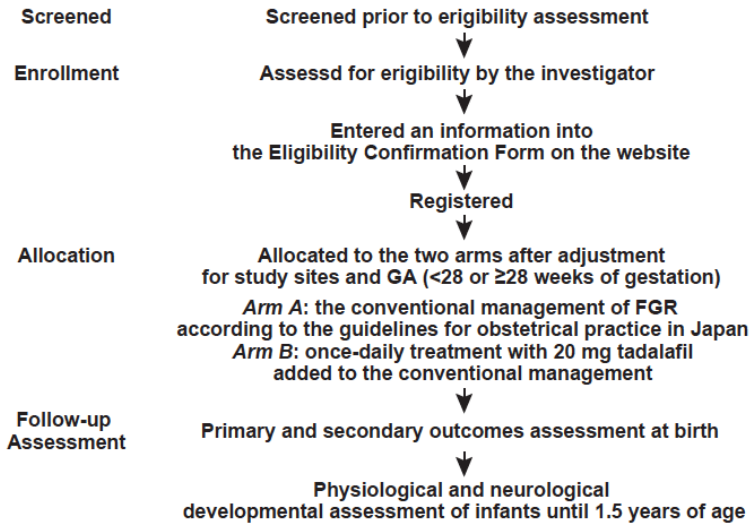

Figure 2. Summary of the study design.

The Clinical Research Support Center in Mie University Hospital will provide data center services including data management and patient registration. Patients that satisfy all inclusion criteria and do not meet any of the exclusion criteria will be eligible for inclusion in the study. Individual study sites will be responsible for guiding potential participants through the informed consent process, including patients who have been referred to them for treatment purposes. The investigator will enter an eligible patient's information into the Eligibility Confirmation Form on the website of this clinical trial (the Clinical Trial Data Management System: Japanese-only website). The data management system will check the contents of the form before registering the patient. For patients who meet all inclusion criteria without violating any of the exclusion criteria listed above, the data management system will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,[1] and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study

sites and GA (<28 or ≥28 weeks of gestation). The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal therapy within 7 days of registration. The investigator will enter the patients' data into the Case Report Form on the website of this clinical trial (the Clinical Trial Data Management System).

The corresponding researcher at Mie University will be responsible for the management of this study (patient registration, data management, and coordination with the study-related committees and the Clinical Research Support Center in Mie University Hospital). The corresponding researcher will also be responsible for the research administration, scheduling, documentation, and safety information management. The Safety Evaluation Committee will assume responsibility for the safety of this study. The Clinical Research Support Center in Mie University Hospital will provide technical support from the planning to the completion of this clinical study. Its Data Management Department will manage the study data in cooperation with the corresponding researcher and secretariats, and its Statistics Department will provide statistical support to facilitate the efficacy evaluation. The Protocol Evaluation Committee is an organization of the execution of this study. All experimental protocols are evaluated and approved by the Protocol Evaluation Committee.

UMIN Clinical Trials Registry UMIN000023778.

Fetal Treatment Protocol

The investigator will provide the fetal therapy as described below.

Arm A: the conventional management of FGR according to the guidelines for obstetrical practice in Japan.[1] Briefly, the conventional management of FGR consists of evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA to evaluate possible pregnancy termination.

Arm B: once-daily treatment with 20 mg tadalafil added to the conventional management until delivery.

The investigators are blinded to the allocation algorithm. Enrolled participants will receive fetal therapy within 7 days of registration.

Rationale for Dose Selection

Tadalafil was approved for treatment of erectile dysfunction (ED) in July 2007 in Japan. Nishiuma S *et al.* reported the results from a post marketing surveillance study on tadalafil, with

a primary goal of confirming the safety and effectiveness of tadalafil in Japanese patients with ED in routine clinical practice. 86.7 % of the participants in the surveillance study were prescribed 10mg or 20mg tadalafil daily.[26] We referred the results of adverse events in the surveillance study and determined the dose of tadalafil in our retrospective study, in which three pregnant women (27.3%) were prescribed 10 mg tadalafil daily and eight pregnant women (72.7%) were prescribed 20 mg daily.[21] In our phase I study, more patients who were administered 40 mg tadalafil daily experienced adverse events than those administered 10 mg or 20 mg tadalafil daily, but we found that there were no serious maternal adverse events.[22] Finally, the minimum required sample size was estimated based on the results of our retrospective study. Taken together, the tadalafil dosage (once-daily treatment with 20 mg) was set in this study.

Stopping Criteria

The investigator must discontinue the protocol-defined treatment when certain events prevent continuation of the protocol treatment. These events include the following:

1. The mother has withdrawn her consent to study participation.
2. Certain events prevent continuation of the protocol treatment, which include the following:

a) A serious adverse drug reaction to tadalafil has developed.

b) The investigator's decision to prioritize other management including termination of the pregnancy instead of continuation of the protocol-defined treatment.

c) The investigator's decision that it is inappropriate to continue with the protocol treatment.

d) The mother's poor compliance or discontinuation of the protocol treatment.

Fetuses in whom the fetal therapy was prematurely discontinued as per the Stopping Criteria will receive scheduled examinations and other assessments to the extent possible. If the mother withdraws her consent to study participation, she and her fetus will be removed from the study. If the fetal therapy is prematurely discontinued due to a serious adverse drug reaction to tadalafil, scheduled subsequent examinations and other assessments should be continued to the extent possible and the investigator should provide the patient experiencing an adverse event with the most appropriate therapeutic measures available. If a registered mother or her fetus is found to have been non-conformant to the eligibility criteria, poor compliance and dropping out with the protocol treatment, the mother or fetus will be categorized as noncompliant.

Criteria for Delivery

In this study, to minimize bias in terms of the timing of delivery, a fetal indication for delivery

is established on the basis of the results from the multicenter survey of VLBW infants in Japan using a network database (Figure 1 and Table 1). After registration, all patients will receive the conventional management of FGR according to the guidelines for obstetrical practice in Japan regardless of the treatment arm.[1] Briefly, the conventional management of FGR consists of the evaluation of fetal well-being on ultrasonography, including Doppler imaging of umbilical arterial blood flow, non-stress test, contraction stress test, and biophysical profile scoring depending on GA, to evaluate possible pregnancy termination. The investigator will evaluate the fetal condition and decide timing of delivery referring to Table 1. For other complications such as preterm labor, rupture of the membranes, and hypertensive disorder of pregnancy, the investigator will follow guidelines for obstetric practice in Japan.[1] The investigator must provide a report that explains the reason for termination of the pregnancy on the website of this clinical trial (the Clinical Trial Data Management System).

Monitoring Safety during the Fetal Therapy

The investigator must pay close attention to the safety of not only the fetus but also the mother. As shown in the study schedule, the protocol-defined assessments include evaluation of maternal blood pressure and pulse rate, maternal blood and urine tests (blood fibrinogen and anti-thrombin III levels, liver and renal function tests, serum electrolyte levels, and qualitative urine protein excretion), maternal serum placental growth factor (PlGF) and soluble fms-like tyrosine kinase receptor (sFLT-1) levels. Other assessments include adverse events assessed by medical consultation, and antepartum fetal tests consisting of ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring depending on GA. The investigator will enter patients' safety data into the Case Report Form on the website of this clinical trial (the Clinical Trial Data Management System).

Safety Evaluation Committee

The Safety Evaluation Committee is responsible for the overall safety of this clinical study. To ensure the safety of the protocol-defined treatment, the Safety Evaluation Committee will review the adverse events of tadalafil treatment. If a serious adverse event develops, the investigator will provide the Secretariat with the necessary information within 24 hours of its onset, according to the predetermined procedure. The Secretariat then will forward the obtained information without delay to the Safety Evaluation Committee for review. The Safety

Evaluation Committee will notify the investigator of the review results. If the adverse event is definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University Hospital or each institute will consider possible termination of this clinical study. Special attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017). Infants will be followed up and evaluated for physiological and neurological development until 1.5 years of age.

Note for New Participating Study Sites

This multicenter study is open to new study sites. It is desirable that study sites cooperate with each other. Agreement on this inter-institutional cooperation is a prerequisite for participation in this clinical study. Case registration requires the approval of the Ethics Committee in each institute.

5. TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY

Based on our previous studies, we do not expect that serious adverse events will occur frequently in this study.[22] However, the investigator may encounter such adverse events as those mentioned in Section 8: Anticipated Adverse Events. The investigator must report adverse drug reactions to the Minister of Health, Labour and Welfare as provided in the Pharmaceuticals and Medical Devices Act. The investigator must also report any serious adverse events without delay to the head of his or her institution, who will in turn forward the information to the Secretariat. The Secretariat will inform the participating study sites of all reported serious adverse events, irrespective of whether expected or unexpected. The Safety Evaluation Committee will review serious adverse event reports and make recommendations to the Principal Investigator, as appropriate. More specifically, the Safety Evaluation Committee will review the information on a serious adverse event that the investigator forwarded as per the predetermined procedure to the Secretariat within 24 hours of its onset. The Safety Evaluation Committee will notify the review results to the investigator. If the adverse event is definitely or probably related to tadalafil treatment, the Ethics Committee in Mie University Hospital or each institute will consider possible termination of this clinical study. Special attention must be paid to the reporting requirements stipulated in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labor, and Welfare in Japan, 2017).

According to the provisions of the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017), the study site will inform the Ministry of Health, Labour and Welfare of unexpected adverse events whose study causality cannot be denied. The Ministry of Health, Labour and Welfare will announce reported serious adverse drug reactions to the public at regular intervals. The study site must provide the patient experiencing an adverse event with the most appropriate therapeutic measures available. In this clinical study, maternal complications associated with the protocol-defined treatment have been covered by liability insurance. However, because fetal complications associated with the protocol-defined treatment have not been covered by liability insurance, the investigator must describe this issue in the informed consent document. The corresponding researcher at Mie University is responsible for dealing with inquiries from participating study sites. In case of an accident, the corresponding researcher will consult the Ethics Committee in Mie University for guidance. This study will comply with the reporting requirements provided in the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017).

6. STUDY PERIOD AND TARGET SAMPLE SIZE

(1) Study Period

The planned study period is from date of ethics approval to February 2021. The Patient Registration Period will last until December 2018. The children's outcome will be followed up for 1.5 years after birth. Data collected by the end of the Neonatal Evaluation Period will be subjected to statistical analysis.

Patient Registration Period: date of ethics approval to December 2018.

Children's Outcome Follow-up Period: 1.5 years after the last birth

(2) Target Sample Size

140 fetuses and their mothers

Rationale for the Target Sample Size

Table 2 shows the summary of the distribution of fetal growth velocity from enrollment to birth in our retrospective study.[21] We estimate that the distribution of fetal growth velocity of this prospective phase II trial will be similar to that of our retrospective study. When the results of our prospective study are analyzed by Wilcoxon Rank Sum Test and group comparisons, with an α of 0.05, two sided, we will have 90% power to detect a difference if we randomize 62 women per group. Allowing for a 10% drop-out rate, the total sample size required is 140 women.

Table 2. The distribution of fetal growth velocity from enrollment to birth in the retrospective study conducted at Mie University Hospital.

Fetal growth velocity (g/day)	<5	≥5 to <10	≥10 to <15	≥15 to <20	≥20 to <25	≥25
The conventional management group (%)	5.3	10.5	21.1	47.3	15.8	0
The tadalafil group (%)	0	8.3	8.3	50.0	16.7	16.7

7. OUTLINE OF THE STUDY PLAN

1. The investigator will register patients with the Clinical Trial Data Management System according to the procedure defined above.

2. The Clinical Trial Data Management System will register and allocate them to the two arms in an allocation ratio of 1:1, one group receiving the conventional management of FGR according to the guidelines for obstetrical practice in Japan,[1] and the other receiving once-daily treatment with 20 mg tadalafil added to conventional management after adjustment for study sites and GA (<28 or ≥28 weeks of gestation).

3. The investigator will conduct the protocol-defined treatment. The Stopping Criteria and the Criteria for Delivery are explained in detail above.

4. Timing and Methods of Evaluation

The investigator will evaluate the variables listed below according to the study schedule. The investigator will use the Case Report Form on the website of this clinical trial (the Clinical Trial Data Management System).

5. Variables

The following safety and efficacy variables will be statistically analyzed:

Variables

(1) Maternal and Fetal

i) Signs and symptoms

Headache, vertigo, flushing, epistaxis, palpitations, anorexia, dyspepsia, diarrhea, nausea, myalgia, arthralgia, dyspnea, and fetal movement counting.

ii) Maternal vital signs

Blood pressure and pulse rate.

iii) Maternal blood and urine test

Complete blood count, blood fibrinogen and anti-thrombin 3 levels, liver and renal function tests, serum electrolyte levels, qualitative urine protein excretion, maternal serum placental growth factor (PlGF), and soluble fms-like tyrosine kinase receptor (sFLT-1) levels.

iv) Fetal ultrasound examination

Estimated fetal weight (g), fetal head circumference (cm), deepest amniotic fluid pocket (cm), Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery)

v) Obstetrics

Onset of obstetrical complications including hypertensive disorder of pregnancy (HDP), treatment for obstetrical complications, indication for delivery, mode of delivery, and placental weight.

vi) Compliance of tadalafil treatment (arm B only).

vi) Adverse events

(2) Neonatal

i) GA at birth.

ii) Physical development

Body weight, height, head circumference, and percentile of birth weight for GA and sex

iii) Apgar score

iv) Clinical laboratory testing

Umbilical artery pH and base excess values

v) Admission in the NICU

vi) Neonatal complications

Respiratory distress syndrome (RDS), pulmonary hemorrhage, neonatal pulmonary hypertension, neonatal chronic lung disease, symptomatic patent ductus arteriosus (PDA), late-onset circulatory dysfunction, intraventricular hemorrhage, periventricular leukomalacia, hypoxic-ischemic encephalopathy, sepsis, necrotizing enterocolitis, gastroesophageal reflux, meconium plug syndrome, retinopathy of prematurity (ROP), anemia of prematurity, auditory disorder (abnormal auditory brainstem response results), congenital abnormality, death, and others.

(3) Pediatric

Physiological and neurological developmental assessment until 1.5 years of age, infant complications including cerebral palsy and epilepsy, and death.

Study Endpoints

(1) Primary endpoint

Fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day).

The primary endpoint is fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), and is calculated using the following formula:

$$\text{Fetal growth velocity (g/day)} = \frac{\text{Birthweight} - \text{EFW at the first day of thetreatment [g]}}{\text{Days of the treatment [days]}}$$

Rationale for the primary endpoint

Our primary hypothesis is that tadalafil therapy will increase the likelihood of increased fetal growth velocity in fetuses with FGR. Taking into account the results of our retrospective study demonstrating that tadalafil treatment increased fetal growth velocity (g/day), which was the primary outcome of the retrospective study, and decreased the incidence of RDS, an improvement in fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day) is an important indicator of the therapeutic benefits for fetuses with FGR.[21]

(2) Secondary endpoints

1) Completion rate of the treatment regimen.

Completion rate of the treatment regimen is defined as the percentage of enrolled patients who receive the protocol-defined treatment for more than 7 days.

2) Efficacy endpoints.

i) Estimated fetal weight (g).

Estimated fetal weight (EFW) is calculated using the following formula:[27]

$$\text{EFW (g)} = 1.07 \times (\text{biparietal diameter: BPD})^3 + 0.3 \times (\text{abdominal circumference: AC})^2 \times (\text{femur length: FL})$$

ii) Fetal growth velocity in the two weeks after the protocol-defined treatment and in the two weeks after one week of the protocol-defined treatment (g/day).

Fetal growth velocity in the two weeks after the protocol-defined treatment (g/day) is calculated using the following formula:

$$\text{Fetal growth velocity in the two weeks after the protocol} - \text{defined treatment (g/day)} = \frac{(\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]})}{14\ [\text{days}]}$$

and fetal growth velocity in the two weeks after one week of the protocol-defined treatment (g/day) is calculated using the following formula:

$$\text{Fetal growth velocity in the two weeks after one week of the treatment (g/day)} = \frac{(\text{EFW three weeks after the treatment} - \text{EFW one week after the treatment [g]})}{14\ [\text{days}]}$$

iii) Fetal growth rate in the two weeks after the protocol-defined treatment and from the first day of the protocol-defined treatment to birth (%/day).

Fetal growth rate in the two weeks after the protocol-defined treatment (%/day) is calculated using the following formula:

$$\text{Fetal growth rate in the two weeks after the protocol-defined treatment (\%/day)} = \frac{\frac{\text{EFW two weeks after the treatment} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100}{14\ [\text{days}]}$$

and Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day) is calculated using the following formula:

$$\text{Fetal growth rate from the first day of the protocol-defined treatment to birth (\%/day)} = \frac{\frac{\text{Birthweight} - \text{EFW at the first day of the treatment [g]}}{\text{EFW at the first day of the treatment [g]}} \times 100}{\text{Days of the treatment [days]}}$$

iv) Fetal head circumference (cm).

The fetal head circumference was measured at the plane of the third ventricle with the thalamus

in the central portion and the cavum septi pellucidi visible in the anterior portion.

v) Doppler imaging of umbilical arterial blood flow.

Umbilical arterial blood flow was examined by Doppler ultrasound according to the Society for Maternal-Fetal Medicine (SMFM) Clinical Guideline.[28]

vi) Deepest amniotic fluid pocket (cm).

The deepest amniotic fluid pocket was measured by transabdominal ultrasonography.

vii) Prolongation of gestational age at birth (days).

Prolongation of gestational age at birth is defined as days from the first day of the protocol-defined treatment to birth.

viii) Birth weight (g).

Birth weight is defined as the weight of the infant at birth.

ix) GA at birth.

GA at birth is defined as the gestational age at birth.

x) Apgar score.

The Apgar score consists of an evaluation of five factors: heart rate, respiratory effort, muscle tone, responsiveness and color at one minute and five minutes after birth.

xi) Umbilical artery pH and base excess values.

Umbilical artery pH and base excess is measured at delivery.

xii) Incidence rate of pre-eclampsia.

Incidence rate of pre-eclampsia is defined as the percentage of enrolled patients who develop pre-eclampsia after the protocol-defined treatment.

xiii) Pediatric developmental assessment until 1.5 years of age.

Pediatric developmental assessment includes physiological and neurological developmental assessment, and infant complications including cerebral palsy, epilepsy, and death.

3) Safety endpoints

i) Incidence rate of obstetric complications.

Incidence rate of obstetric complications including HDP is defined as the percentage of enrolled patients who develop obstetric complications after the protocol-defined treatment.

ii) Perinatal mortality.

Perinatal mortality is defined to include stillbirths (occurring after 22 weeks of gestation) and neonatal deaths (occurring up to 7 days after birth).

iii) Neonatal mortality.

Neonatal mortality is defined as neonatal deaths occurring up to 28 days after birth.

(3) Statistics

Analysis is done on all randomized fetuses who receive the protocol-defined treatment at least once, as the full analysis set. Analysis per protocol set (i.e., removing patients who do not meet the inclusion and exclusion criteria) is done as a secondary analysis population for sensitivity analysis. All outcome measures are presented as summaries of descriptive statistics (mean [SD] or median [minimum and maximum] for continuous measures, and the numbers and proportions for ordinal and dichotomous measures). Descriptive statistics for the primary endpoint, i.e. fetal growth velocity from the first day of the protocol-defined treatment to birth (g/day), are analyzed for each treatment arm by the Wilcoxon Rank Sum Test and group comparisons. All analyses are performed according to a pre-specified statistical analysis plan. The Data Coordinating Center in Mie University Hospital supports the data management, statistical analysis, and reporting of the study.

8. ANTICIPATED ADVERSE EVENTS

Because we have already demonstrated in phase I clinical trial that tadalafil treatment was feasible in pregnant women with FGR,[22] tadalafil treatment for FGR can be administered with relative safety and ease. Yet, this therapy may give rise to unexpected adverse events, given the limited clinical experience with this approach and exposure of healthy mothers without pulmonary hypertension to tadalafil. The investigator must fully inform prospective participants of such possibility and administer the fetal therapy with careful attention and monitoring. Adverse reactions to tadalafil divided into the four groups by the frequency (Very common [≥1/10], common [≥1/100 to <1/10], uncommon [≥1/1,000 to <1/100], and not known [cannot be estimated from the available data]) described in the product information of tadalafil (ADCIRCA®20 mg tablets) are shown below:[29]

- Very common (≥1/10)

  Headache, flushing, nasopharyngitis, nausea, dyspepsia, myalgia, nack pain, and pain in extremity.

- Common (≥1/100 to <1/10)

  Hypersensitivity reactions*, syncope, migraine*, blurred vision, palpitations*, ***, hypotension, epistaxis, vomiting, gastroesophageal reflux, rash, increased uterine bleeding**, facial oedema, and chest pain***.

- Uncommon (≥1/1,000 to <1/100)

Seizures*, transient amnesia*, tinnitus, Sudden cardiac death*,***, Tachycardia*,***, hypertension, urticaria*, hyperhydrosis*, haematuria, priapism*, penile haemorrhage, and haematospermia

- Not known (cannot be estimated from the available data)

  Angioedema, stroke***, non-arteritic anterior ischemic optic neuropathy, retinal vascular occlusion, visual, field defect, sudden hearing loss, unstable angina pectoris, ventricular arrhythmia, myocardial infarction***, Stevens-Johnson Syndrome, exfoliative dermatitis, and prolonged erections.

* The adverse reactions have been included in the table as a result of postmarketing or clinical study data from the use of tadalafil in the treatment of erectile dysfunction; and in addition, the frequency estimates are based on only 1 or 2 patients experiencing the adverse reaction in the pivotal placebo controlled study of ADCIRCA®.

** Clinical non-Medical Dictionary for Regulatory Activities (MedDRA) term to include reports of abnormal/excessive menstrual bleeding, conditions such as menorrhagia, metrorrhagia, menometrorrhagia, or vaginal hemorrhage.

***Most of the patients in whom these events have been reported had pre-existing cardiovascular risk factors.

9. POTENTIAL BENEFITS AND RISKS

(1) Benefits

Potential benefits of this study include cure or improvement in FGR.

(2) Risks

Maternal exposure to tadalafil is inevitable in patients allocated tadalafil treatment arm. Therefore, precautions must ensure the safety of both the mother and the fetus. Specific descriptions of such risks have been described in Section 8: Anticipated Adverse Events. To control for such risks, this study has stipulated an array of tests, such as hematology, serum chemistry, medical consultation, and antepartum fetal tests consisting of ultrasonography including Doppler imaging of blood flow (umbilical artery, middle cerebral artery, and uterine artery), non-stress test, contraction stress test, and biophysical profile scoring depending on GA. In the event of an adverse drug reaction, the investigator will immediately take appropriate measures, possibly including early withdrawal from the study. The investigator must prioritize maternal safety over fetal therapy. If the mother develops an adverse drug reaction, it will be treated under liability insurance and / or the national health insurance scheme.

10. BURDEN OF COST

This research was supported by by the Japan Agency for Medical Research and Development (AMED). This fund will be paid for items related to research (purchasing cost for tadalafil, data management, storage, analysis, etc.) other than medical examination. Medical examination expenses are covered by the national health insurance scheme.

11. INTELLECTUAL PROPERTY RIGHTS

Any intellectual property rights that may arise from this clinical study shall be exclusively owned by the TADAFER study group. The corresponding researcher and the joint researchers report no conflicts of interest related to this clinical study or to their organizations.

12. ETHICS

This clinical study focuses on prenatal treatment, and its protocol has been developed according to the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare in Japan, 2017). Before the start of this clinical study, the corresponding researcher will explain its objectives and outline them fully to the participating site investigators. We believe that application of the guideline requirements to the mother who consents to participate in this study will ensure that her fetus is also protected by the ethical principles of the guidelines. As per the Ethical Guidelines for Clinical Studies, participation in this study will be preceded by the informed consent process. Considering the difficulty in obtaining assent, even implicitly, from the fetus, we believe that the parental permission for the fetus to participate.

13. ADDITIONAL NOTES RELATED TO THE ACT ON THE PROTECTION OF PERSONAL INFORMATION RELATED BY INDEPENDENT ADMINISTRATIVE INSTITUTIONS

1. Data Collection

Study data will be de-identified before being stored in electronic format. De-identified or anonymous data will be analyzed at Mie University. Joint researchers will examine and discuss the analyzed results.

2. Data Management

The results of analyses of the collected test data will be securely stored at the Secretariat located in Mie University.

3. Storage of Electronic Media

The results of analyses will be filed in electronic media, which will be kept securely in a locked room of Mie University. The Secretariat staff member, Dr. Takashi Umekawa, assumes the responsibility for data storage. In addition to the corresponding researcher, appointed members of the Secretariat staff will be granted access to the study data.

4. Method and Timing of Data De-identification

Registration numbers will be used to de-identify the study data at individual study sites. Each study site must ensure that the data they transfer to the Secretariat contains no explicit personal identifiers.

5. Notification of Analytical Results

Parents who participate in this study will not be informed of the results of this study.

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