



BMJ Open Tadalafil treatment for fetuses with early-onset growth restriction: a protocol for a multicentre, randomised, placebo-controlled, double-blind phase II trial (TADAFER Iib)

Shintaro Maki ¹, Hiroaki Tanaka,¹ Sho Takakura,¹ Masafumi Nii,¹ Kayo Tanaka,¹ Toru Ogura,² Mayumi Kotera,² Yuki Nishimura,² Satoshi Tamaru,² Takafumi Ushida,³ Yasuhiro Tanaka,⁴ Norihiko Kikuchi,⁴ Tadatsugu Kinjo,⁵ Hiroshi Kawamura,⁶ Mayumi Takano,⁷ Koji Nakamura,⁸ Sachie Suga,⁹ Michi Kasai,¹⁰ Osamu Yasui,¹¹ Kenji Nagao,¹² Yuka Maegawa,¹³ Tomomi Kotani,³ Masayuki Endo,⁸ Ichiro Yasuhi,⁹ Shigeru Aoki,¹⁰ Yoichi Aoki,⁵ Yoshio Yoshida,⁶ Masahiko Nakata ⁷, Akihiko Sekizawa,¹¹ Tomoaki Ikeda¹

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For numbered affiliations see end of article.

Correspondence to

Dr Shintaro Maki;
s-maki@med.mie-u.ac.jp

ABSTRACT

Introduction The TADAFER I treatment for Fetuses with early-onset growth Restriction: multicentre, randomised, phase II trial (TADAFER II) study showed the possibility of prolonging the pregnancy period in cases of early-onset fetal growth restriction; however, it was an open-label study. To establish further evidence for the efficacy of tadalafil in this setting, we planned a multicentre, randomised, placebo-controlled, double-blind trial.

Methods and analysis This trial will be conducted in 180 fetuses with fetal growth restriction enrolled from medical centres in Japan; their mothers will be randomised into three groups: arm A, receiving two times per day placebo; arm B, receiving one time per day 20 mg tadalafil and one time per day placebo and arm C, receiving 20 mg two times per day tadalafil. The primary endpoint is the prolongation of gestational age at birth, defined as days from the first day of the protocol-defined treatment to birth. To minimise bias in terms of fetal baseline conditions and timing of delivery, a fetal indication for delivery as in TADAFER II will be established in this trial. The investigator will evaluate fetal baseline conditions at enrolment and decide the timing of delivery based on this indication.

Ethics and dissemination This study has been approved by Mie University Hospital Clinical Research Review Board on 22 July 2019 (S2018-007). Written informed consent will be obtained from all mothers before recruitment. Our findings will be widely disseminated through peer-reviewed publications.

Trial registration jRCTs041190065.

INTRODUCTION

Fetal growth restriction (FGR) is a common condition in pregnancy caused by various factors such as diabetes mellitus, autoimmune disease, hypertensive disorders of

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This is the first randomised, placebo-controlled, double-blind trial to prospectively evaluate the efficacy and safety of tadalafil treatment in fetuses with fetal growth restriction (FGR), for which there is no proven therapy.
- ⇒ Participants will be enrolled from major medical centres in Japan that provide treatment for fetuses with FGR according to the guidelines for obstetrical practice in Japan.
- ⇒ To minimise bias in terms of fetal baseline conditions and delivery timing, a fetal indication for delivery was established in this study based on the results of a multicentre survey in Japan.
- ⇒ The inclusion criteria (estimated fetal weight ≤ -1.5 SD) include not only cases originating from placental insufficiency but also a certain number of small for gestational age cases. It is thought that severe FGR is untreatable and our criteria include early stages of FGR for which tadalafil may have efficacy.
- ⇒ This study will be conducted up to the neonatal period (4 weeks after birth) and will not include a study of the long-term prognosis of the child. The long-term prognosis will be evaluated in a separate study.

pregnancy, antiphospholipid antibody syndrome, infection, genetic and structural disorders.¹⁻⁴ It is associated with various adverse outcomes and increased perinatal mortality.⁵⁻¹² Furthermore, chronic hypoxia and malnutrition may increase the risk of future developmental disorders such as autism, learning disorders and lifestyle-related diseases such as hypertension and type 2 diabetes.^{13 14} However, there is no

radical treatment for this serious condition, except for ensuring delivery at an appropriate time such that the condition does not become unmanageable.¹⁵

One of the likely FGR treatments currently being studied is phosphodiesterase (PDE) 5 inhibitors. The Sildenafil Therapy In Dismal Prognosis Early-Onset Intrauterine Growth Restriction (STRIDER) consortium investigated sildenafil, a PDE5 inhibitor, as a potential treatment for FGR; however, the studies did not show improvement of the perinatal outcomes.^{16–23}

Tadalafil, another PDE5 inhibitor, has a long half-life and high selectivity for PDE5^{24 25}; however, its effects on the fetus via the placenta differ from those of sildenafil, another PDE5 inhibitor.²⁶ We hypothesised that tadalafil could improve fetoplacental perfusion in placental insufficiency caused by aforementioned factors. We investigated tadalafil's therapeutic effect against FGR in retrospective, phase I and phase II studies.^{27–29}

The phase I trial for establishing the safety of administration to the maternal body for FGR treatment was performed in 2015.²⁸ The trial had an open-label design and involved daily administration of 10, 20 and 40 mg of tadalafil in 12 cases. Mild headache, palpitation and facial flushing were frequently observed as maternal adverse events in this trial. However, no tadalafil-related severe maternal or neonatal adverse event was observed in these patients.

As the next step, a multicentre, randomised, controlled phase II trial (TADAlafil treatment for Fetuses with Early-onset growth Restriction: TADAFER II) was conducted at a major medical centre in Japan.²⁹ Following the STRIDER UK paper, which reported no benefits of sildenafil for pregnancy prolongation, was published in February 2018.¹⁷ Our grant sponsor, the Japan Agency for Medical Research and Development, concluded that all PDE5 inhibitors are ineffective against FGR due to the STRIDER-UK study results and recommended that we cease recruiting new candidates for the TADAFER II trial by the end of March 2018. Accordingly, we ceased recruiting and analysed the data of the 89 cases registered at that time, mainly for the safety of tadalafil treatment and protocol-defined outcomes. The safety of mothers and fetuses and the prolongation of pregnancy, which suggest the potential effect of tadalafil treatment for FGR, were observed in the results. Tadalafil has different effects from sildenafil in terms of placental transfer²⁶ and PDE5 selectivity,²⁵ and we consider it to have different effects on FGR. However, TADAFER II was an incomplete study; therefore, we have not been able to prove the efficacy of tadalafil, but we supposed it could be effective. Because TADAFER II had an open-label design, to establish further evidence of efficacy, we planned a multicentre, randomised, placebo-controlled, double-blind trial.

Tadalafil has a Tmax of 3 hours a day, after which the blood concentration drops, so we considered that taking 20 mg two times a day would stabilise the blood concentration. Therefore, this study was designated as a phase

II 'exploratory study' in the sense of dose determination. Our plan is that the results of this study will lead to a phase III confirmatory study.

METHODS AND ANALYSIS

Study design

This is a multicentre, randomised, placebo-controlled, double-blind trial.

Study period

The planned study period extends from the date of ethical approval (22 July 2019) to 31 May 2023. The patient registration period extends until 31 May 2022, starting from 1 October 2019. Data collection will be performed until 4 weeks after the birth of the children enrolled. Data collected by the end of the neonatal evaluation period will be subjected to statistical analyses.

Patient selection

Subjects are mothers whose fetuses have FGRs. The inclusion criteria are as follows: (1) pregnant women aged ≥ 20 years, < 45 years; (2) estimated fetal weight (EFW) of -1.5 SD or less of the mean EFW for gestational age (GA) according to the Japanese standard curve³⁰; (3) GA between 20+0 and 31+6 weeks; (4) expected date of confinement determined using the criteria of the guidelines for obstetrical practice in Japan (2017); (5) singleton pregnant women and (6) provision of signed written informed consent from the pregnant women.

The exclusion criteria were as follows: (1) antepartum fetal tests performed at enrolment indicating that delivery should be attempted, (2) a history of allergy to tadalafil, (3) use of concurrent medications that interact adversely with tadalafil, (4) contraindication of tadalafil treatment due to renal disease and liver diseases, uncontrolled arrhythmia, hypertension (BP: $> 170/100$ mm Hg) or hypotension (BP: $< 80/40$ mm Hg), (5) fetus with suspected chromosomal disorder and/or multiple congenital anomalies, (6) contraindication of tadalafil due to retinitis pigmentosa, coagulation defect, active gastric and/or intestinal ulcer or venous obstructive disease and (7) study entry deemed inappropriate by the investigators.

Intervention information

The protocol allows for mothers to be either inpatient or outpatient. Fetal therapy will be administered as follows. The conventional management of FGR according to the guidelines for obstetrical practice in Japan will be followed for all trial patients.³¹ Briefly, it consists of an evaluation of fetal well-being by ultrasonography, including Doppler velocimetry of umbilical arterial blood flow, non-stress and contract stress tests and biophysical profile scoring depending on GA to evaluate possible pregnancy termination.^{31 32}

Arm A: patients will be provided with two times per day placebo along with the conventional management until delivery.

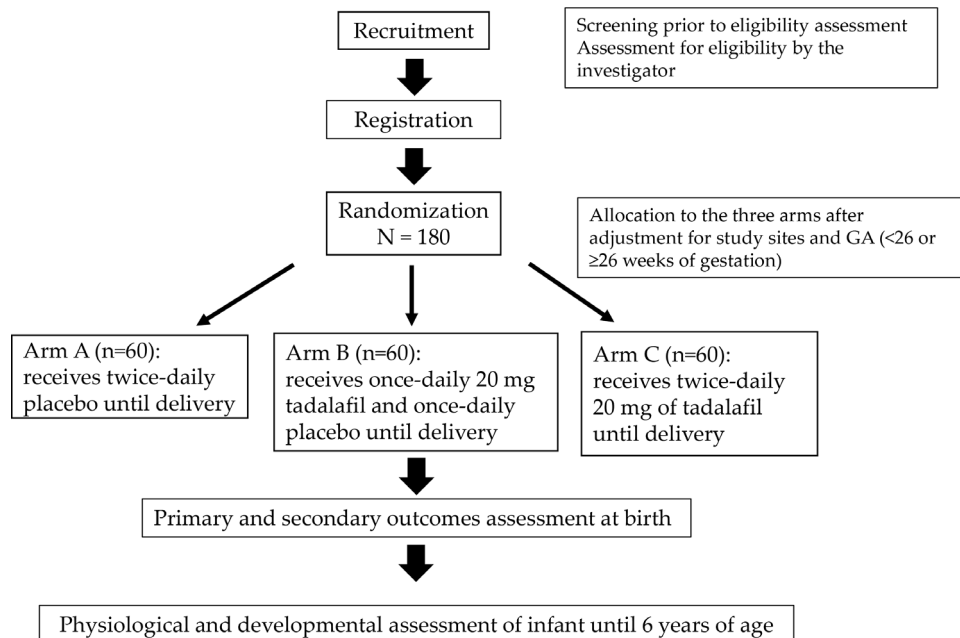


Figure 1 The flowchart of this trial. GA, gestational age.

Arm B: patients will be provided one time per day 20 mg tadalafil and one time per day placebo (morning: tadalafil, evening: placebo) along with the conventional management until delivery.

Arm C: patients will be provided two times per day 20 mg tadalafil (total 40 mg) along with the conventional management until delivery.

The test drug for this trial is powdered medicine. The time of administration is clearly marked on the package of the test drug (8:00 or 20:00), if there is any missed or wrong dose, it will be recorded in a diary, and the number of extra drugs will be checked. Investigators, clinicians and other staff in charge of participants are blinded to the allocation algorithm and the consequence. Enrolled participants will receive fetal therapy within 3 days of registration.

Registration and randomisation

The study protocol defines all the procedures and schedules by which the investigators must abide, including patient selection and registration, fetal treatment of FGR and follow-up (figure 1). Patients who satisfy the inclusion criteria and do not meet any of the exclusion criteria will be eligible for inclusion. Individual study sites will be responsible for guiding potential participants through the informed consent process, including patients referred to them for treatment purposes. After acquiring patient consent, an investigator will fill the registration form and send it to the Clinical Research Support Center at Mie University. The patient will be allocated to one of the three trial arms. The investigators will be blinded to the allocation algorithm. The random sequence will be generated by the process of minimisation. Randomisation will be performed using a modified Pocock and Simon's minimisation method, with the same rate for each group,

considering the GA (<26 or 26 weeks) and facility of enrolment as adjustment factors. The random sequence generation and randomisation will be performed by a computer programme. Allocation information will be provided only to an unblinded drug administrator, and the test drug for each arm will be prepared. The investigators, patients, staff involved in treatment will be blinded to the allocation and informed only of the numbers assigned in the order of enrolment. Enrolled participants will receive fetal therapy within 3 days of registration.

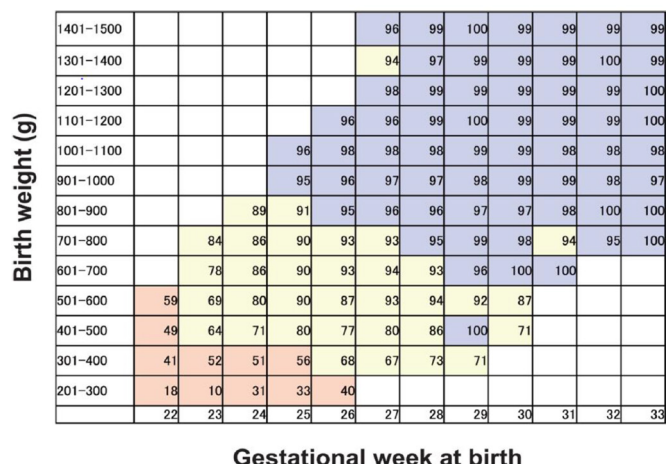
Delivery criteria

To minimise bias in terms of fetal baseline condition at enrolment, a fetal indication for delivery was established based on the results of a multicentre survey of very low birth-weight infants in Japan using a network database, in which 82 level-III perinatal centres were registered. The survey data included infant survival rate in the neonatal intensive care unit (NICU), categorised by birth weight and the gestational week at birth (figure 2).³³ The infant survival rate data acquired from the survey were pre-processed using the moving average method and divided into three groups. The first group is defined as 'zone 1', where the infant survival rate in the NICU is <60%. The second group is defined as 'zone 2', where the infant survival rate in the NICU is 60%–95%. The third group is defined as 'zone 3', where the infant survival rate in the NICU is ≥95% (table 1).

Discontinuation criteria

Investigators must discontinue the protocol-defined treatment in the following situations:

1. The mother withdraws her consent for trial participation.



Gestational week at birth

Figure 2 Infant survival rate in the neonatal intensive care unit (NICU) categorised according to birth weight and gestational week at birth (%). This figure has been prepared based on the results from the multicentre survey of very-low-birth-weight infants in Japan by using a network database. The survey data include infant survival rates in the NICU, categorised by birth weight and gestational week at birth. The infant survival rate data acquired from the survey were preprocessed with the moving average method and divided into three groups. The first group is defined as 'zone 1', where the infant survival rate in the NICU is <60% (highlighted by the red background). The second group is defined as 'zone 2', where the infant survival rate in the NICU is 60% to 95% (highlighted by the yellow background). The third group is defined as 'zone 3', where the infant survival rate in the NICU is ≥95% (highlighted by the blue background).

2. Certain events prevent the continuation of the treatment protocol, which include the following:
 - A serious adverse drug reaction to tadalafil.
 - Decision by the investigator that it is inappropriate to continue with the treatment protocol.

- Poor compliance by the mother or discontinuation of the treatment protocol.

Data collection

Study entry information

- ▶ Date of randomisation.
- ▶ Date of starting the protocol-defined treatment.

Maternal characteristics at registration

- ▶ Maternal age.
- ▶ Maternal height.
- ▶ Maternal body weight at registration.
- ▶ Parity.
- ▶ Smoking status.
- ▶ Maternal history associated with FGR.

Collagen disease: antiphospholipid antibody syndrome; systemic lupus erythematosus; other collagen diseases; blood disorders; hyperthyroidism or hypothyroidism and a history of FGR, hypertensive disorder of pregnancy, diabetes and kidney disease.

- ▶ Other maternal history (free description).
- ▶ Maternal obstetric complications.
- ▶ Fetal ultrasonography assessment at registration.

EFW; head circumference; abdominal circumference; maximum vertical pocket and Doppler study of the umbilical artery, middle cerebral and uterine arteries, ductus venosus and umbilical vein

- ▶ Use of aspirin or magnesium sulfate.
- ▶ Estimated date of delivery.
- ▶ Laboratory data (complete blood count and coagulation and biochemical tests).

Primary endpoint

The primary endpoint is the prolongation of GA, defined as days from the first day of the protocol-defined treatment to birth. In the case of intrauterine fetal demise, the date on which death is confirmed is the day of delivery.

Table 1 Fetal indication for delivery of fetuses with early-onset growth restriction: the TADAFER IIb study

Infant survival rate in the neonatal intensive care unit (NICU) (see figure 1)	A fetal indication for delivery
Zone 1	Decide the timing of delivery depending on therapeutic measures available at the NICU in each institute.
Zone 2	Consider delivery if at least one of the following three findings is noted. <ol style="list-style-type: none"> 1. Reversed umbilical artery blood flow during diastole. 2. Score lower than six on the fetal biophysical profile scoring. 3. Fetal heart rate patterns in the orange or red category for more than 30 min
Zone 3	Consider delivery if at least one of the following five findings is noted. <ol style="list-style-type: none"> 1. Reversed or absent umbilical artery blood flow during diastole. 2. Score lower than six on the fetal biophysical profile scoring (score lower than eight on the fetal biophysical profile scoring if oligohydramnios is present). 3. Fetal heart rate patterns in the orange or red category for more than 30 min. 4. Positive contraction stress test. 5. Impaired fetal head circumference growth for more than 2 weeks.

TADAFER IIb: Tadalafil treatment for fetuses with early-onset growth restriction: multicentre, randomised, placebo-controlled, double-blind phase II trial

Comparison of arms A and B and arms A and C.

Secondary endpoints

1. Completion rate of the treatment regimen

The 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 and who can receive more than 90% of the medicine.

2. Prolongation of GA at birth

Comparison of groups B and C.

3. Efficacy monitoring

3.1 Kaplan-Meier plot of the interval from the start of protocol-defined treatment to birth.

3.2 EFW

EFW is calculated using the following formula according to the guidelines of the Japan Society of Obstetrics and Gynecology considering the local chart³⁴:

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

3.3 Fetal growth velocity

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

3.4 Fetal growth velocity in the 2 weeks after the protocol-defined treatment

The fetal growth velocity in the 2 weeks after the protocol-defined treatment (g/day) is calculated using the following formula:

$$\text{Fetal growth velocity in the 2 weeks after the protocol - defined treatment (g/day)} = \frac{\text{EFW 2 weeks after the treatment} - \text{EFW on the first day of the treatment [g]}}{14 \text{ [days]}}$$

3.5 Fetal growth rate in the 2 weeks after the protocol-defined treatment and from the first day of the protocol-defined treatment to birth

Fetal growth rate in the 2 weeks after the protocol-defined treatment (%/day) is calculated using the following formula:

Fetal growth rate in the 2 weeks after the protocol-defined treatment (%/day)

$$\frac{\text{EFW 2 weeks after the treatment} - \text{EFW on the first day of the treatment [g]} \times 100}{\text{EFW on the first day of the treatment [g]} \times 14 \text{ [days]}}$$

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

Fetal growth rate from the first day of the protocol-defined treatment to birth (%/day)

$$\frac{\text{Birth weight EFW on the first day of the treatment [g]} \times 100}{\text{EFW on the first day of the treatment [g]} \times \text{Days of the treatment [days]}}$$

3.6 Fetal head circumference.

3.7 Fetal abdominal circumference.

3.8 Doppler imaging of umbilical arterial blood flow, middle cerebral and uterine arteries and ductus venosus.

3.9 Deepest amniotic fluid pocket or the deepest value of amniotic fluid index (AFI).

3.10 Birth weight of newborns.

3.11 SD of birth weight of newborns.

3.12 Birth height of newborns.

3.13 Gender of newborns.

3.14 GA at birth.

3.15 Rate of caesarean section or vaginal delivery.

3.16 Maternal blood pressure and heart rate.

3.17 Apgar score (1 min and 5 min).

3.18 Umbilical artery pH and base excess values.

3.19 Incidence rate of pre-eclampsia and days from the start of treatment to the onset.

3.20 Analysis of maternal urine.

3.21 Maternal serum placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1).

3.22 Neonatal morbidity.

3.23 Assessment of neonate at birth.

The data of fetal biometric parameters (EFW, head circumference, abdominal circumference) at the start of protocol-defined treatment (allowed up to 3 days), 1 (± 2 days) and 2 weeks (± 2 days) after the start of protocol-defined treatment and at the time of delivery ($- 7$ days) will be analysed as secondary outcomes.

The data for the Doppler study and maximum vertical pocket at the start of treatment (allowed up to 3 days) and 3 days ($+1$ day), 1 (± 2 days) and 2 weeks (± 2 days), and at the time of delivery (7 days) after the start of treatment will be analysed.

The data for maternal heart rate, blood pressure at registration, the start of treatment, day 3 ($+1$ day), 1 (± 2 days) and 2 weeks (± 2 days) and at the time of delivery (7 days) after the start of treatment will be analysed. In addition, the data for urinary protein at registration, day 3 ($+1$ day) and 1 week (± 2 days) after the start of treatment will be analysed.

We defined the neonatal period to be within 4 weeks after birth. Neonatal morbidity is defined as the risk of neonatal death or serious conditions due to immaturity, necrotising enterocolitis, retinopathy of prematurity, intraventricular haemorrhage, periventricular leukomalacia, hypoxic-ischaemic encephalopathy, neonatal respiratory distress syndrome, chronic lung disease, persistent pulmonary hypertension, pulmonary haemorrhage, patent ductus arteriosus, surgical patent ductus arteriosus (PDA) ligation, anaemia of prematurity, meconium plug syndrome, sepsis, gastro-oesophageal reflux disease and congenital anomaly. Oxygen, nitric oxide (NO), indomethacin and surfactant use as related treatments, as well as other neonatal diseases listed, will be investigated.

4. Safety monitoring

4.1 Incidence rate of obstetric complications.

4.2 Incidence rate of intrauterine demise.

4.3 Perinatal mortality.

4.4 Neonatal mortality.

4.5 Maternal adverse event.

All symptoms presented by the mother, whether or not caused by tadalafil, will be considered adverse events.

The main symptoms that can be caused by tadalafil include headache, facial flushing, palpitations, anorexia, dizziness, muscle pain, nasal haemorrhage

and vomiting, which will be enumerated and aggregated. In addition, any other symptoms presented by the mother will be collected as free entries.

Statistical analysis plan

The primary endpoint will be analysed on an intention-to-treat (ITT) basis. The analysis will include all randomised participants for whom outcome data are available. The analysis of secondary endpoints is the main analysis targeting the full analysis set (FAS), which will include all randomised fetuses who receive the protocol-defined treatment at least once. Furthermore, the per-protocol set (PPS) will be analysed to confirm analysis stability. The safety endpoint analysis targets the safety analysis set (SAF). Each analysis will be performed by trial statisticians at Mie University. The subject of each set is described as follows:

ITT: the analysis of all registered cases.

FAS, SAF: (1) Patients who meet the inclusion criteria and do not meet the exclusion criteria.

(2) Patients who receive the protocol treatment more than once.

PPS: (1) patients without protocol deviation.

(2) Patients who receive the protocol treatment for more than 1 week.

Sample size

One hundred and eighty fetuses and their mothers. Each arm will include 60 cases.

Rationale for the target sample size

Regarding limiting cases registered at <32 weeks in the TADAFER II study, significant prolongation of the days from the start of the protocol-defined treatment till delivery in the tadalafil treatment group compared with that in the conventional treatment group was observed. Thus, we decided that the primary endpoint of the study is the prolongation of GA at birth defined as the days from the start of the protocol-defined treatment till delivery. A comparison of the prolongation of GA between groups will be made. The clinically relevant difference in the primary endpoint is 7 days, which is considered to be associated with improving infant survival rate in NICU and neonatal morbidity.

The target sample size was calculated based on the results of TADAFER II. The days from the start of the protocol-defined treatment to delivery in the cases registered at less

than 32 gestational weeks calculated based on the results of the TADAFER II study are summarised in [table 2](#) for the tadalafil 20 mg oral administration and conventional treatment groups.

In the study, arms A and B will receive the same treatments as the conventional treatment and tadalafil 20 mg oral administration groups of the TADAFER II, respectively. Hence, it is assumed that the duration of pregnancy is a close value. To date, there has been no report on the administration of 40 mg tadalafil for FGR with regards to the period of pregnancy. Therefore, the pregnancy period of arm C (40 mg tadalafil) was assumed to be similar to that of arm B because it is unlikely that it would be shorter than that of the tadalafil 20 mg group. When the results of our prospective study are analysed using Wilcoxon rank-sum test and group comparisons (first, comparing arms A and B, and then, comparing arms A and C), with a significance level of 5%, two sided, we will have 80% power to detect a difference if 56 women are randomised to each group. In the TADAFER II trial, three patients (3.4%) dropped out. With reference to the results, 180 cases were set as the total number of cases, with 60 cases in each group.

Significance level

The two-sided significance level was set at 0.05. Because the trial involves a three-arm comparison, the first test will be performed in the order of two predetermined groups (eg, arms A and B) by a two-sided significance level of 0.05 in a fixed sequence test.³⁵ If the first test shows a significant difference, the next test of two groups (eg, arms A and C) will be performed. If the significance is not detected, further tests are not performed. In the multiple comparison test among the three arms using the Bonferroni method, the two-sided significance level is 1.67%.

Statistical approach for endpoints

Characteristics at registration

Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables will be used for unadjusted analyses (the two-sided significance level is set to 1.67% using the Bonferroni method).

Primary endpoint

In the ITT and PPS analyses, the median and quartile values will be calculated for each group. Intergroup comparisons will be performed using the Wilcoxon

Table 2 Distribution of the prolongation of GA defined as the days from the start of the protocol-defined treatment to delivery based on the results of the TADAFER II trial

Prolongation of GA (days)	~9	10~19	20~29	30~39	40~49	50~59	60~69	70~79	80~89	90~99	100~
Tadalafil treatment group	2 (6%)	4 (13%)	3 (9%)	2 (6%)	4 (13%)	4 (13%)	3 (9%)	4 (13%)	3 (9%)	2 (6%)	1 (3%)
Conventional treatment group	6 (18%)	5 (15%)	4 (12%)	5 (15%)	3 (9%)	3 (9%)	2 (6%)	3 (9%)	0 (0%)	1 (3%)	1 (3%)

TADAFER II: Tadalafil treatment for fetuses with early-onset growth restriction: multicentre, randomised, phase II trial
GA, gestational age.

rank-sum test with a fixed sequence approach. The tests will be performed in the following order.

1. Comparison of arms A and B (significance level: 5%)
2. Comparison of arms A and C (significance level: 5%)

If a significant difference is found in the comparison of arms A and B and no significant difference is found between arms A and C, significance exists only for arms A and B.

The analysis will be performed after the deliveries of all registered cases.

Secondary endpoints

For the FAS and PPS, the Wilcoxon rank-sum test or unpaired t-test will be used for the ratio and interval scales with a fixed sequence approach. Fisher's exact test will be used for the nominal scale with a fixed sequence approach. Regarding GA prolongation, Kaplan-Meier curves for each of the two groups will be drawn, and the generalised Wilcoxon test will be performed with a two-sided significance level of 5%.

Subgroup analysis

The cases will be divided into GA subgroups at registration below 30, 28, 26 and 24 weeks or more. Subgroup analysis based on the Doppler measurements at inclusion (umbilical artery pulsatility index >95th centile, absent-reversed end-diastolic flow in the umbilical artery) will also be performed. All endpoints will be analysed in these subgroups.

Safety evaluation

Information on adverse events that occurred during the trial in terms of the kind of event, outcome, severity, causal relationship and comments will be collected and reported by the investigators. An adverse event is defined as any unfavourable and unintended sign, symptom or disease occurring during the study period without any judgement about causality or relationship to the drug. Special attention will be paid to the reporting requirements stipulated in the Clinical Trial Act (Ministry of Health, Labour and Welfare in Japan, 2018). In case of a serious adverse event listed below, the investigator belonging to each medical centre will report it within 24 hours to the principal investigator or research secretariat.

1. Life-threatening effect.
2. Requires hospitalisation or prolonged hospitalisation for treatment (hospitalisation for FGR or delivery is not included).
3. Causes permanent or significant disability or malfunction.
4. Causes congenital abnormalities in the offspring.

In addition, unblinding will be considered in the following cases.

1. When a serious adverse event related to the life or death of the mother occurs.
2. When the number of fetal death cases exceeds 20% of all registered cases.

3. When the number of neonatal death cases exceeds 20% of all registered cases.

To ensure the safety of the protocol-defined treatment, Mie University Hospital Clinical Research Review Board (Certified Review Board) or the safety evaluation committee will review the adverse events of tadalafil treatment. If the adverse event is probably or definitely related to tadalafil, the Clinical Research Review Board or Safety Evaluation Committee (composed of medical doctors in the fields of obstetrics and gynaecology and paediatrics) will consider possible termination of the trial. Other non-serious adverse events are reviewed in a regular annual report in a blinded manner. In addition, infants will be followed-up and evaluated for physiological and neurological development until 6 years of age in a separate study.

Interim analysis

An interim analysis will be performed to assess safety. When the number of the registered cases reaches 90, fetal and neonatal deaths will be tabulated for each group, and if they exceed 40%, case registration will be ceased. The interim analysis results must not be communicated to any organisations or individuals other than the Safety Evaluation Committee, as this may affect the conduct and evaluation of the trial.

Follow-up of children

We have prepared the follow-up protocol for the children of mothers participating in the study, which will be conducted at each facility. At the time of participation in this study, investigators will explain to the patients the follow-up of children, and consent will be obtained.

The follow-up survey will be performed at 1, 3–4, 6–7 and 9–10 months; 1, 1.5 and 2 years of corrected age and 3, 4, 5 and 6 years of age. The neonatal outcome at discharge from the hospital will also be included in the survey. The checked items according to each age will be investigated. Although the survey will be carried out by visit, it will also be acceptable by telephone. The Kyoto Scale of Psychological Development will be performed at 1.5 years of corrected age and 3 years of age. In addition, the Wechsler intelligence scale for children at the age of 6 will be performed at each facility.

Participating institutions

Mie University, Nagoya University, Shinshu University, Ryukyu University, Fukui University, Toho University, Osaka University, National Hospital Organization Nagasaki Medical Center, Yokohama City University Medical Center, Showa University, Municipal Yokkaichi Hospital, and Mie Chuo Medical Center.

Patient and public involvement

Patients and/or the public were not involved in the design, recruitment or conduct of this trial.

ETHICS AND DISSEMINATION

The trial was approved by the Mie University Hospital Clinical Research Review Board on 22 July 2019 (S2018-007)



before patient enrolment, complies with the Helsinki Declaration, and has been registered in the Japan Registry of Clinical Trials (<https://jrct.niph.go.jp/latest-detail/jRCTs041190065>). Written informed consent will be obtained from all mothers before recruitment. Our findings will be widely disseminated through conference presentations and peer-reviewed publications.

DISCUSSION

Double blinding is important to avoid biases as much as possible,³⁶ and this is a multicentre, randomised, placebo-controlled, double-blind trial. Since TADAFER II had an open-label design, as the next step, a placebo-controlled, double-blind trial is needed to establish further evidence of tadalafil efficacy in FGR management. The placebo was developed in collaboration with the Pharmaceutical Department at Mie University, and high blindness was confirmed.

Tadalafil is a specific and long-acting PDE5 inhibitor whose effect on the fetus differs from that of sildenafil. Walton *et al* reported that sildenafil citrate reversed precontracted placental–fetal arterial perfusion in an ex-vivo human placental model, whereas tadalafil produced no response.²⁶ This means that the difference in the effects between tadalafil and sildenafil in the fetus via the placenta might result in differences in safety and efficacy. The Dutch STRIDER group reported an increased risk of neonatal pulmonary hypertension due to maternal sildenafil administration.²⁰ In contrast with the incidence, tadalafil is safe in terms of fetal and neonatal morbidity, which was not significantly different between the tadalafil treatment and control groups.²⁹

The primary endpoint of this trial is GA prolongation. The sample size required was estimated based on the results of the TADAFER II trial. In the TADAFER II trial, tadalafil significantly prolonged the pregnancy period, limiting cases in which tadalafil was administered at <32 weeks. Therefore, with reference to the results of the TADAFER II trial, the participants of the present trial would be determined as having received tadalafil treatment at <32 weeks of GA, and GA prolongation was determined to be the primary endpoint. The same delivery criteria as those in the TADAFER II trial have been adopted in this trial, and the accuracy of the study endpoint will be ensured by strictly adhering to the criteria.

Fetuses with an EFW of -1.5 SD or less of the mean EFW for GA will be included in this trial. The inclusion criteria have been preliminarily established according to the Japanese obstetrical guidelines and the results of TADAFER II. Although these criteria include a given number of physiologically normal fetuses that are simply small for GA (SGA) and less severe cases derived from placental insufficiency, a previous but incomplete study provided positive data on GA prolongation by including these cases.²⁹ In the STRIDER UK study, the participants were in advanced stages of FGR with both size and Doppler

abnormalities necessary for inclusion, indicating the study was focused on a much more severe type of FGR. It is thought that severe FGR is untreatable. Our criteria include early stages of FGR; thus, there is a possibility the condition could be treated before it becomes irreversible. We have previously shown that tadalafil is safe for use in mothers, fetuses and newborns and can be administered in less severe cases prophylactically.^{28 29}

This study will be conducted up to the neonatal period (4 weeks after birth) and does not include a study of the long-term prognosis of the child. It has been reported that SGA children have more cognitive challenges, inattention-hyperactivity symptoms and school challenges than appropriate-for-GA children.³⁷ While it is important to evaluate neonatal outcomes in the short term, it is also important to examine the impact of tadalafil on neurological outcomes in the long term. Therefore, the long-term prognosis of tadalafil-treated children will be evaluated in a separate study.

Author affiliations

¹Department of Obstetrics and Gynecology, Mie University Graduate School of Medicine, Tsu, Mie, Japan

²Clinical Research Support Center, Mie University Hospital, Tsu, Mie, Japan

³Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan

⁴Department of Obstetrics and Gynecology, Shinshu University Graduate School of Medicine, Matsumoto, Nagano, Japan

⁵Department of Obstetrics and Gynecology, University of the Ryukyus, Nakagami-gun, Okinawa, Japan

⁶Department of Obstetrics and Gynecology, Fukui University Graduate School of Medicine, Fukui, Japan

⁷Department of Obstetrics and Gynecology, Toho University Faculty of Medicine, Otaku, Tokyo, Japan

⁸Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

⁹Department of Obstetrics and Gynecology, National Hospital Organization Nagasaki Medical Center, Omura, Nagasaki, Japan

¹⁰Perinatal Center for Maternity and Neonate, Yokohama City University Medical Center, Yokohama, Kanagawa, Japan

¹¹Department of Obstetrics and Gynecology, Showa University Graduate School of Medicine, Shinagawa-ku, Tokyo, Japan

¹²Department of Obstetrics and Gynecology, Yokkaichi Municipal Hospital, Yokkaichi, Mie, Japan

¹³Department of Obstetrics and Gynecology, Mie Chuo Medical Center, Tsu, Mie, Japan

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ORCID iDs

Shintaro Maki <http://orcid.org/0000-0001-9194-8013>

Masahiko Nakata <http://orcid.org/0000-0002-3621-1251>

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