Two-dimensional phase-contrast magnetic resonance imaging reveals changes in uterine arterial blood flow in pregnant women treated with tadalafil for fetal growth restriction: A prospective study.

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Abstract

Objective: To examine the effect of uterine arterial (UtA) blood flow changes after tadalafil treatment for fetal growth restriction (FGR) using two-dimensional (2D) phase-contrast magnetic resonance imaging (PC-MRI). Design: Prospective, controlled study. Setting: Single tertiary center. Population or Sample: Fourteen pregnant women with FGR aged 20–44 years and at [?]20 weeks’ gestation were recruited between May 2019 and July 2020. Methods: They underwent 2D PC-MRI for UtA blood flow measurement 3 days after diagnosis. They were compared with 14 gestational age (GA)-matched healthy pregnant women (control group). Six patients in the FGR group received tadalafil at 20 mg twice daily after the first MRI until delivery. They underwent a second MRI 1 week later and were compared with 6 of the 14 GA-matched healthy pregnant women. Main Outcome Measures: Total UtA blood/body surface area Results: The median total UtA blood/body surface area was 420 mL/min/m² (290–494) in the FGR group and 547 mL/min/m² (433–681) in the control group (p=0.01). Percent increases of blood flow in the FGR and control groups were significantly different at 68.8% (51–75) and 18.8% (16–57), respectively (p<0.01). Both intraobserver reproducibility (r=0.997, p<0.01; intraclass correlation coefficients (ICC), 0.997) and interobserver reproducibility (r=0.997, p<0.01; ICC, 0.998) were high. Conclusions: UtA blood flow in pregnant women with FGR was significantly lower than that in healthy pregnant women. Tadalafil may improve UtA blood flow, thereby improving placental function in pregnant patients with FGR.

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Conclusions: UtA blood flow in pregnant women with FGR was significantly lower than that in healthy pregnant women. Tadalafil may improve UtA blood flow, thereby improving placental function in pregnant patients with FGR.

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Keywords: uterine arterial blood flow, fetal growth restriction, phase-contrast magnetic resonance imaging, tadalafil treatment, placental insufficiency

Introduction

Fetal growth restriction (FGR) is often the result of one or more maternal, placental, or fetal disorders that interfere with the normal mechanisms regulating fetal growth. Other than fetal disorders, specific etiology in FGR includes 1) maternal factors such as hypoxemia, anemia, and environmental factors, 2) placental factors such as maternal vascular malperfusion pathology (infarction, fibrin deposition, chronic abruption), chronic placental inflammation, and chronic abruption and 3) umbilical cord factors such as increased coiling, increased cord length, and single umbilical artery. Suboptimal uteroplacental perfusion of fetal nutrition is involved in these factors, sometimes leading to decreased blood flow to the fetus and stagnation of oxygen supply, thereby worsening fetal outcomes.\(^1\)\(^-\)\(^3\) Chronic hypoxia and malnutrition increase the risk of not only preterm birth and perinatal death but also short stature and neurodevelopmental disorders such as mental retardation, attention deficit hyperactivity disorder, autism spectrum disorder, learning disabilities after birth, and lifestyle-related diseases such as hypertension, obesity, and impaired glucose tolerance in adulthood.\(^4\)\(^-\)\(^9\)

However, effective treatment for FGR remains unknown. Currently, in medical practice, a fetus is delivered prematurely before circulatory failure or brain growth retardation becomes apparent and then switched to out-of-utero treatment in the neonatal intensive care unit.
Recently, a novel treatment method for pregnant women with FGR complications has been developed by orally administering tadalafil, a phosphodiesterase-5 (PDE-5) inhibitor and potent vasodilator. Tadalafil selectively inhibits PDE-5 expressed in vascular smooth muscles, degrades cyclic guanosine monophosphate (cGMP), decreases intracellular cGMP and Ca^{2+} concentrations, and dilates vascular smooth muscles in uterine spiral arteries, which supposedly improves oxygen and nutrient supply to the fetus. However, the effect of tadalafil on human uterine arterial (UtA) blood flow remains unknown.

Two-dimensional (2D) phase-contrast magnetic resonance imaging (PC-MRI) is a noninvasive imaging technique for quantifying arterial blood flow. 2D PC-MRI enables the measurement of arterial blood flow not only in the great arteries but also in the small arteries, such as the coronary sinus and coronary arteries, with high reproducibility. This technique could be applied to the measurement of blood flow in the uterine artery, but its usefulness in this area has not been established. Herein, we aimed to measure UtA blood flow using 2D PC-MRI in pregnant women with FGR and normal pregnant women and examine whether the results 1) adequately indicate the association of dysfunctional placenta with decreased uterine blood flow and 2) show how UtA blood flow would be affected by tadalafil administration.

**Methods**

**Study population**

A total of 15 pregnant women with FGR who provided informed consent were prospectively recruited in this cross-sectional study between May 28, 2019, and July 29, 2020. FGR is defined as fetal growth less than 1.5 standard deviations (SDs) below the mean estimated fetal body weight (EFBW) according to GA as calculated via ultrasonography (Voluson E8Expert, GE Healthcare, Zipf, Austria), per Japanese standards. FGR was diagnosed by an obstetrician proficient in fetal ultrasound procedures in our facility. The GA of the fetus was calculated per the mother’s last menstrual period date or by considering the crown-rump length in the first trimester if a discrepancy in dates exceeded 7 days. Eligible patients were aged 20–44 years at their last normal menstrual period, with a healthy singleton pregnancy of ≥20 weeks’ gestation. Exclusion criteria were as follows: (1) threatened preterm labor with difficulty in undergoing MRI, (2) MRI contraindications such as metallic implants or devices and claustrophobia, (3) insufficient MRI scan quality, and (4) informed consent not provided. Of the 15 pregnant women with FGR, 1 was excluded because she was transferred to another hospital. The remaining 14 women with FGR underwent PC-MRI 3 days (2-4) after diagnosis. This group (FGR group) was compared with 14 GA-matched healthy pregnant women (control group) (Analysis 1). Treatment with tadalafil was initiated after the first MRI for six patients in the FGR group. They were administered 20 mg of tadalafil twice daily (40 mg per day), and administration was continued until delivery. Tadalafil has a Tmax of 3–4 hours a day, after which the blood concentration reduces; hence, we considered that taking 20 mg twice daily would stabilize the blood concentration. In dose-repeat administration studies, plasma concentrations have been found to reach a steady state on the fifth day of oral administration. Therefore, the six tadalafil-treated patients underwent a second 2D PC-MRI approximately 1 week later, 4 hours after the evening oral dose. Further, this group was compared with six GA-matched healthy pregnant women of the 14 healthy pregnant women (controls) (Analysis 2). Figure 1 is an overview of the study’s participant flowchart.

**MRI**

MRI studies were performed in accordance with International Society of Ultrasound in Obstetrics and Gynecology Practice Guidelines using a 3.0 T MR scanner (Ingenia 3.0 Ω, Philips Healthcare, Best, The Netherlands) using dS coils for signal reception. No sedative medications or contrast agents were used. All participants underwent MRI in the half left lateral decubitus position to minimize aortocaval compression. Initially, non-gated three-dimensional time of flight (TOF) MR angiography covering the maternal pelvis from the abdominal bifurcation of the aorta to the pubic symphysis was performed to allow localization of both the left and right uterine arteries (repetition time [TR]/echo time [TE]/flip angle [FA] = 25 ms/3.45 ms/18°, field of view [FOV]/rectangular FOV/scan percentage = 340 mm/100%/55%, scan matrix/reconstruction matrix = 308/640, section number/section thickness/section overlap = 190/1.6 mm/50%, compressed SENSE factor
= 5, vein saturation slab thickness/gap = 31.8 mm/10 mm, tilted optimized non-saturating excitation pulse with a central flip angle = 18°, actual bandwidth = 216.4 Hz/pixel, measured voxel size = 0.92×1.32×1.6 mm³, reconstructed voxel size = 0.53×0.53×0.8 mm³, and number of signals averaged [NSA] = 1). Thin slice multiplanar reconstruction (MPR) images of the TOF pelvic MR angiogram were generated on the MR console in three orthogonal directions (trans axial, sagittal, and coronal) to determine the cross-sectional imaging plane of the proximal portion of the right and left uterine arteries.

To measure uterine blood flow, peripherally gated 2D PC-MR images were acquired on the imaging plane perpendicular to the proximal portion of the right and left uterine arteries by separately referring to the MPR images (TR/TE/FA = 4.8 ms/3.2 ms/10°, FOV/rectangular FOV/scan percentage = 300 mm/86.1%/100%, scan matrix/reconstruction matrix = 144/288, slice thickness = 8 mm, SENSE factor = 1, actual bandwidth = 723.4 Hz/pixel, measured voxel size = 2.08×2.15×8 mm³, reconstructed voxel size = 1.04×1.04×0.8 mm³, NSA= 1, reconstructed frames per cardiac cycle = 25, and velocity encoding [VENC] = ±80–150 cm/s). VENC was optimized for each uterine artery so that it was not less than but as close as possible to the maximal blood velocity in the uterine arteries of each patient. PC-MRI was repeated five times using optimal VENC for the right and left uterine arteries in each patient. Including magnet setup and patient preparation, the entire imaging protocol was completed in approximately 30–45 min. (Figure S1)

MR image analysis

PC-MR images were analyzed using a commercial workstation (EWS, Philips Healthcare) by two independent observers blinded to the GA and obstetric history of the study participants. The contour of the uterine artery was manually traced on the magnitude images at each cine frame. The traced region of interest was applied to the corresponding phase image, and the cross-sectional area and mean velocity were recorded. Volumetric UtA blood flow (mL/min) was calculated by integrating the product of cross-sectional area and mean velocity in the uterine artery from the 25 images acquired across the cardiac cycle.

The volumetric blood flow in each uterine artery was calculated by averaging the results from five repeated scans. For Analyses 1 and 2, UtA blood flow was measured five times using PC-MRI by imaging the left and right uterine arteries in each case. The sum of the left and right arteries was taken as the total UtA blood flow, and the mean value of the five imaging sessions was reported as the total UtA blood flow value for each case. Total UtA blood flow was indexed to BSA. In Analysis 2, UtA blood flow was measured twice in the pregnant women with FGR and in the healthy pregnant women at the same GA, which corresponded to measurements before and 1 week after the administration of tadalafil in the pregnant women with FGR (measurements 1 and 2, respectively). Percent increase of UtA blood flow was calculated between measurements 1 and 2 in both groups. In addition, one of the two observers measured the changes in the uterine arteries twice 3 months apart to determine the intraobserver reproducibility.

Statistical analysis

Statistical analyses were performed using SPSS Statistics 20.0 (IBM, Armonk, NY, USA). Continuous variables are reported as medians (interquartile ranges). Categorical values are presented as numbers (percentages). Comparison between variables was performed using the chi-square test or Fisher’s exact test for categorical variables and the Wilcoxon’s signed rank test for continuous variables. Intraobserver and interobserver assessments of PC-MRI flow variables were reported using Spearman’s rank correlation coefficient analysis, Bland–Altman plots and intraclass correlation coefficients (ICCs). In all analyses, p<0.05 indicated statistical significance. However, a significant linear correlation between two variables was defined when both p<0.05 and |r| > 0.2 were met. We considered r values between ±0.40 and ±0.59 to indicate a moderate correlation, those between ±0.60 and ±0.79 to indicate a strong correlation, and those greater than ±0.80 to indicate a very strong correlation.

Results

Characteristics of participants

Characteristics of the pregnant women with FGR and the healthy pregnant women are summarized in
Table 1. Pregnant women with FGR and healthy pregnant women were similar in age, at 34 years (29–39) and 33 years (30–36), respectively (p=0.91); body mass index (BMI), at 20.0 kg/m² (17.7–21.7) and 20.3 kg/m² (19.1–22.6), respectively (p=0.54); body surface area (BSA) calculated using the Du Bois & Du Bois formula, at 1.53 m² (1.50–1.60) and 1.60 m² (1.53–1.64), respectively (p=0.15); heart rate, at 71 beats per minute (bpm) (65–74) and 79 bpm (65–86), respectively (p=0.11); and systolic blood pressure, at 110 mmHg (104–117) and 110 mmHg (104–118), respectively (p=0.75). The proportions of nulliparous women and those with in vitro fertilization-induced pregnancy were not significantly different respectively. MR examination was performed at gestational days 214 (202–235) and 211 (200–235) for the second and third trimesters of pregnancy, respectively (p=0.80). The FGR and control groups were significantly different in SD of EFBW at MRI, at -2.2 (-2.4–-2.1) and 0 (-0.3–0.5) (p<0.01); gestational days at delivery, at 261 (248–270) and 272 (269–278) (p<0.01); fetal birth weight, at 1880 g (1416–2158) and 2974 g (2752–3289) (p<0.01); and placental weight, at 381 g (300–418) and 558 g (466–710) (p<0.01), respectively. Neonatal deaths were not observed in either group.

Characteristics of the six patients with FGR who received tadalafil administration and the six women with normal pregnancy (controls) are summarized in Table 2. Between the two groups, only BSA was significantly different (p=0.03), whereas age and BMI were not different (p=0.42 and p=0.87, respectively). The median of gestational days on the tadalafil administration initiation date was 211 (204–223) days. Gestational days at the time of the first MRI and number of days between the first and second MRI were similar at 215 days (207–228) and 214 days (206–227) (p=0.82) and 6.5 days (6–7) and 6.5 days (6–7) (p=0.82) between pregnant women with FGR and healthy controls, respectively.

UtA blood flow in pregnant women with FGR and in healthy pregnant women (Analysis 1)
Total UtA blood/BSA was 420 mL/min/m² (290–494) in the FGR group and 547 mL/min/m² (433–681) in the control group (p=0.01) (Figure S2).

UtA blood flow in pregnant women with FGR who received tadalafil treatment and in healthy pregnant women (Analysis 2)
On comparing the change in UtA blood flow in patients with FGR who received tadalafil treatment and in healthy pregnant women between measurements 1 and 2, we found a significant increase in blood flow/BSA in both groups, from 367 mL/min/m² (290–487) to 437 mL/min/m² (345–570) (p=0.03) and from 548 mL/min/m² (456–614) to 574 mL/min/m² (476–672) (p=0.03), respectively (Figure 2).

To examine the effect of tadalafil treatment on UtA blood flow, we compared the percent increase of UtA blood flow in both groups and found a significant difference between the pregnant women with FGR treated with tadalafil and healthy pregnant women (68.8% (51–75), 18.8% (16.0–57), p<0.01, respectively), as shown in Figure 2. In addition, the fetal weight gain in the FGR group treated with tadalafil was similar to that in the control group, from 1236 g (1033–1306) to 1324 g (1213–1418) (p=0.03) and from 1500 g (1306–1772) to 1617 g (1385–1997) (p=0.03), respectively (Figure S3).

Intraobserver and interobserver assessments of PC-MRI flow measurements
Linear regression of measurements and Bland–Altman analysis of measurement 1 and measurement 2 (intraobserver) and measurements of observer 1 and observer 2 (interobserver) are presented in Figure S3. The intraobserver correlation was very strong (r=0.997, p<0.01). Bland–Altman plot analysis for intraobserver correlation revealed the following results: bias, 4.9; SD of bias, 11.5; 95% limits of agreement, -17.7 to 27.6; and ICC, 0.997. The interobserver correlation was also very strong (r=0.997, [p<0.01]). Bland–Altman plot analysis for interobserver correlation revealed the following results: bias, -6.5; SD of bias, 10.4; 95% limits of agreement, -26.9 to 14.0; and ICC, 0.998 (Figure S4).

Discussion

Main Findings
This study revealed two major findings. First, pregnant women with FGR had significantly lower UtA blood flow than healthy pregnant women. Second, in the tadalafil-treated FGR group, the UtA blood flow increased more than that in controls.

Strengths and Limitations

The strength of our study lies in demonstrating the feasibility of measuring uterine arterial blood flow using 2D PC-MRI, which has traditionally been challenging. We further investigated the effect of tadalafil on FGR using this approach that ensured high intra- and interobserver reproducibility. This robust methodology increases the reliability of our findings. Furthermore, the technique we used has the potential to evaluate the effects of other drugs. This study has some limitations. First, the sample size was small for the pregnant women with tadalafil treatment in this study. Those patients were recruited from a single center during a short period between phase II and phase IIb clinical trials. As the phase IIb clinical trial is underway at the moment, we are unable to recruit further patients into this study. Second, the diagnosis of FGR in the present study followed the Japanese criteria. Globally, FGR without congenital anomalies is determined using the Delhi procedure. Nonetheless, 13 of the 14 patients (93%) recruited in this study met this criterion.

Interpretation

In this study, pregnant women with FGR had significantly lower UtA blood flow than controls. Of the few studies evaluating abnormal pregnancy using MRI, Hwuang reported that total UtA blood flow decreased in small for gestational age (SGA) infants and women with preeclampsia and that this result was linked to poor pregnancy outcomes. Konje et al. reported that women who delivered FGR infants had 12.5% and 36.7% lower UtA blood flow than that of healthy controls at 20 and 38 weeks, respectively, using the ultrasound technique. Thus, the results of our study and previous studies support a relationship between the decrease in UtA blood flow and the retardation of fetal growth. However, the mechanisms underlying such a relationship remain unknown. A previous study demonstrated that UtA blood flow assessed using Doppler ultrasound and O₂ delivery estimated using blood samples were two-fold greater at 20 weeks gestational age in Andean women genetically adapted to high altitudes than in European women at high altitudes and that those increases in UtA blood flow and O₂ delivery were paralleled by greater fetal sizes. This observation leads to the hypothesis that preserved UtA blood flow during pregnancy might play a role in protecting fetal O₂ supply and growth. We previously demonstrated that placental oxygenation was significantly reduced in the FGR group than in controls. As efficient oxygen extraction requires sufficient fetal blood flow in the intervillus and chorionic villi, it is likely that fetal blood flow and chorionic villus changes in FGR reduce oxygen extraction efficiency. Additionally, the days to delivery were significantly shorter in the FGR group than in the controls in the present study; this might be due to insufficient blood flow between the uterus and placenta owing to placental abnormalities. Therefore, placental insufficiency is considered a cause of preterm delivery. Thus, measurement of UtA blood flow might allow for early detection of FGR and associated poor pregnancy outcomes such as preterm birth. Further studies are warranted in this regard.

Tadalafil-treated FGR patients showed an increase in EFBW and UtA blood flow more than those in the controls. The mechanism of action of tadalafil is supposedly dilation of vascular smooth muscles in the uterine spiral artery, leading to placental vasodilatation and improved oxygen and nutrient supply to the fetus, and our results are consistent with this theory. We demonstrated that tadalafil improved the width of maternal blood sinuses in the labyrinth zone of the placenta in a mouse model of preeclampsia but did not significantly alter fetal capillaries. Sekimoto et al. revealed that a murine reduced uterine perfusion pressure model showed an increased sFlt-1 protein level in the placenta, and tadalafil corrected it to control levels. It probably decreases vascular resistance in the placental vascular bed and increases blood flow, leading to improved placental circulation and reduction in placental ischemia. As a result, they showed that tadalafil improved placental hypoxia through corrected enhanced HIF1a expression. However, sildenafil citrate improved pre-constricted placental arterial perfusion in a human placental model, whereas tadalafil showed no response. Possibly, tadalafil does not cross the human placental barrier or is degraded by trophoblasts. This proves that tadalafil is safe for the fetus; Maki et al. also reported that tadalafil reduced fetal and FGR-related
infant deaths and significantly prolonged gestational length in patients with GA < 32 weeks. Improvement in placental insufficiency owing to increased UtA blood flow with tadalafil administration might have contributed to these results.

The present study demonstrated that the inter- and intraobserver errors were very small. MRI is a non-invasive modality, free from radiation exposure, and allows for the objective evaluation of the uterine artery blood flow without the administration of gadolinium contrast medium, even in patients difficult to examine with ultrasound examination. Hwuang et al. reported the possibility of using 4D-flow MRI performed at 3T to assess the anatomy and hemodynamics of uterine arteries in women in the second and third trimesters of pregnancy. While their method has been proven to be highly reproducible, the study by Sussman et al. evaluated uterine arteries with 2D PC-MRI more accurately than with 4D-flow MRI. Our method can be considered useful in that it was found to be as good as or better than the modality of Sussman et al. in reproducibility.

**Conclusion**

UtA blood flow in pregnant women with FGR was significantly lower than that in healthy pregnant women. Tadalafil improved UtA blood flow in pregnant women with FGR, which is expected to extend the number of weeks of gestation and improve placental function in these patients. A placebo-controlled randomized controlled trial as a multicenter-validation clinical trial (Phase IIb) is underway, the results of which are awaited.

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**Disclosure of Interests**

None

**Contribution to Authorship**

MN participated in study design, scored the studies, conducted the statistical analysis, interpreted the data, and drafted the manuscript. NE scored the studies, conducted the statistical analysis, MI participated in study design, scored the studies, interpreted the data, and revised the manuscript. SM, ST, SM, KT, KT, HT and EK interpreted the data. HS participated in study design, interpreted the data, and revised the manuscript. TI conceived the study, participated in its design, interpreted the data, and revised the manuscript.

All authors read and approved the final manuscript.

**Details of Ethics Approval**

This prospective study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Clinical Research Ethics Review Committee of Mie University Hospital (H2019-075, May 17, 2019). All women provided their written, informed consent prior to participation in this study.

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**References**


Figure legends

Figure 1.
Study flow chart. FGR, fetal growth restriction; GA, gestational age.

Figure 2.
Amount and rate of change in UtA blood flow between the first and second magnetic resonance imaging examinations. UtA, uterine artery.

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Table_1.docx available at https://authorea.com/users/631626/articles/650867-two-dimensional-phase-contrast-magnetic-resonance-imaging-reveals-changes-in-uterine-arterial-
blood-flow-in-pregnant-women-treated-with-tadalafil-for-fetal-growth-restriction-a-prospective-study

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