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Comparative determination of placental perfusion by magnetic resonance imaging and contrast-enhanced ultrasound in a murine model of intrauterine growth restriction

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ABSTRACT

Introduction: Exploration of placental perfusion is essential in screening for dysfunctions impairing fetal growth. The objective of this study was to assess the potential value of contrast-enhanced ultrasonography (CEUS) and magnetic resonance imaging (MRI) for examining placental perfusion in a murine model of intrauterine growth restriction (IUGR). We also studied the reproducibility of perfusion quantification by CEUS.
Methods: Pregnant Sprague Dawley rat models of IUGR were studied during the third trimester. Unilateral uterine artery ligation induced IUGR. Placental perfusion was evaluated by CEUS and perfusion MRI with gadolinium for both ligated and control fetoplacental units. The kinetic parameters of the two imaging modalities were then compared.
Results: The analysis included 20 rats. The study showed good reproducibility of the CEUS indicators. The CEUS perfusion index approximated the blood flow rate and was halved in the ligation group (27.9 [u.a] (± 14.8)) versus 61 [u.a] (± 22.3) on the control side ($P = 0.0003$). MRI with gadolinium injection showed a clear reduction in the blood flow rate to 51.2 mL/min/100 mL (IQR 34.9–54.9) in the ligated horn, compared with 90.9 mL/min/100 mL (IQR 85.1–95.7) for the control side ($P < 0.0001$). The semiquantitative indicators obtained from the kinetic curves for both CEUS and MRI showed similar trends. Nonetheless, values were more widely dispersed with CEUS than MRI.
Discussion: The similar results for the quantification of placental perfusion by MRI and CEUS reinforce the likelihood that CEUS can be used to identify IUGR in a murine model induced by uterine vessel ligation.

1. Introduction

Fetoplacental vascularization, because it is critical for the exchange of oxygen and nutrients between maternal and fetal tissue, determines fetal growth. Intrauterine growth restriction (IUGR) is defined as the failure of the fetus to achieve its genetically determined growth potential [1]. One of its main causes is impaired spiral arterial remodeling [2], which compromises uterine circulation in the placenta and is an important feature that predicts fetal risk of perinatal morbidity and long-term sequelae, in particular, clusters of cardiovascular risk factors in childhood [3].

Ultrasound imaging and especially Doppler ultrasound have been applied to assess both the umbilicoplacental and uteroplacental circulations. Ultrasound estimates of fetal growth together with measurements of vascular resistance by uterine Doppler currently allow the diagnosis of IUGR, albeit with low sensitivity, which ranges from 15 to 60%, and a high false-positive rate — 30% [4,5]. Fetal perfusion is evaluated with the cerebroplacental ratio (CPR), deduced by Doppler measurements of the cerebral and umbilical artery resistance index values. This ratio makes it possible to assess the fetus's hemodynamic

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tolerance and to adapt obstetric management appropriately. Nonetheless, some animal studies have found that in situations of chronic hypoxia this ratio is normalized as fetal oxygen consumption falls [6,7]. This adaptive phenomenon is thus an important limitation on the diagnostic use of the CPR. These flaws of Doppler ultrasound tools make it difficult to predict the optimal time to deliver IUGR fetuses.

Different methods of noninvasive imaging of perfusion can be used to quantify the maternal placental vascularization: magnetic resonance imaging (MRI) and contrast-enhanced ultrasonography (CEUS). CEUS has been used extensively to measure blood flow in many organ systems and is performed [8] by the acoustic detection of microbubble contrast agents that remain within the intravascular compartment. The enhancement of the signal obtained makes it possible to increase the echogenicity of blood vessels and thus to visualize the microcirculation. Some studies have already shown that the increase in placental perfusion during the course of gestation can be quantified [9,10]. MRI has also been applied to evaluate changes in tissue perfusion, oxygenation, and permeability associated with placental function disorders [11]. Regional tissue perfusion can be assessed with MRI after administration of gadolinium-based contrast agents or with noncontrast imaging approaches that apply arterial spin labeling [12]. Analysis of time-intensity curves from these two imaging modalities allows researchers to deduce information about the placental vascular flow and thus to better understand pathological situations.

The objective of our study was to assess the potential value of these two imaging methods for estimating placental perfusion on the maternal side of a murine model of IUGR induced by ligation of the uterine vascular pedicle. We also assessed the reproducibility of the CEUS measurements.

2. Material and methods

2.1. Animals

The ethics committee of the Centre-Val de Loire region and the Ministry of Agriculture approved the procedures used, all of which met with national and European guidelines for the well-being of animals.

Rats were all pregnant Sprague Dawley (CERJ, Le Genest saint-Isle, France) animals, aged 10–12 weeks. The first day of the gestation was defined as the day after mating. The rats were fed *ad libitum*, with 12-h day/night cycles in the facility's animal rooms. After arrival, all rats had an acclimation period of seven days. The surgical and imaging procedures took place under anesthesia by mask induction of isoflurane (Vetflurane®). The physical temperature was maintained at 37 °C by laboratory hot plates.

2.2. IUGR model

The IUGR model was induced by unilateral ligation of the uterine pedicle on day 17 of gestation, as described by Wigglesworth in 1964 [13], leading to placental hypovascularization, which causes fetal IUGR. The model was validated after cesarean delivery on day 20 of gestation, by weighing the rat fetuses from each uterine horn and observing a weight difference of at least 20% between the rat fetuses from the ligated and control horns.

The rats were then divided into two groups for imaging: CEUS and MRI. These separate groups were set up for several different reasons. First, the effects of the consecutive injection of the two different contrast agents, one for ultrasound and another for MRI, is not known. Moreover, the performance of two different imaging protocols would have doubled the anesthesia time. Finally, the animal's position differs in each of these imaging modalities, a difference that prevented a strictly comparable examination of the same placenta by two techniques.

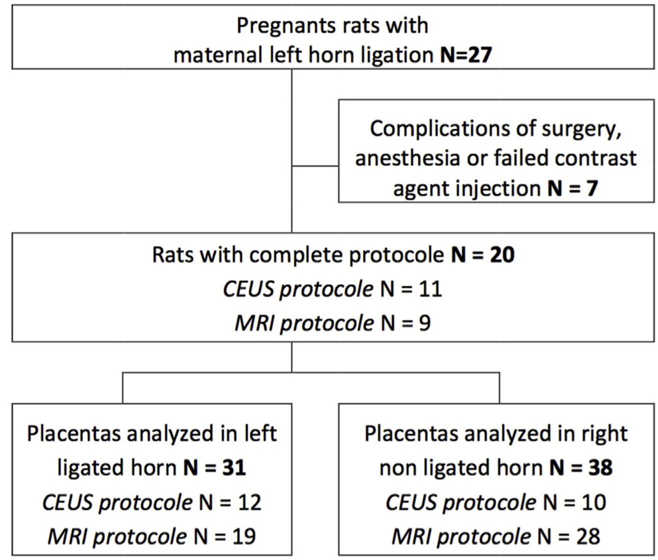

Fig. 1. Flow chart.

2.3. CEUS procedure

The contrast-enhanced ultrasound scans took place on day 19 of gestation with a Vevo2100 instrument (Visualsonics Inc., Toronto, Canada), connected to a 21-MHz probe (MS250). The instrument settings were the same for all acquisitions. The ultrasound contrast agents used were Definity microbubbles® (Lantheus Medical Imaging; Billerica, MA; 200 µL (1 mL/kg) diluted in NaCl 0.9%), injected into the rat's caudal vein; these are known to remain in the vascular compartment. After correction for respiratory movements, the analysis was performed in bolus mode with VevoCQ software (Visualsonics Inc, Toronto, Canada). Microbubbles were injected a maximum of 4 times at intervals at least 20 min apart; they never exceeded 10% of the rat's blood volume (10–13 mL).

The various quantitative indicators described are the maximum signal intensity (PE: Peak Enhancement), time to maximum intensity (TTP: Time to Peak), speed of contrast uptake (WiR: Wash-In Rate), and blood volume (WiAUC: area under the curve between T_0, defined by the injection of contrast agents, and TTP). To study the wash-out rate (WoR), kinetic curves were extrapolated with Matlab when necessary. To determine quantitative indicators, regions of interest were manually designed and included the whole placenta of interest on either the ligated or the non-ligated side.

The intra- and inter-observer reproducibility of the CEUS measurements performed by two operators (C.A. and V.M.) was studied for 60 measurements of each parameter. The analysis followed the guidelines for studying reproducibility (*Guidelines for Reporting Reliability and Agreement Studies*) [14].

2.4. MRI procedure

The MRI acquisitions were performed after the injection of the contrast agent into the caudal vein on day 19 of gestation, with a 9.4-Tesla horizontal imaging system (Bruker Biospin, Wissembourg, France). The anesthetized rats were positioned on a high-definition 72-mm homogeneous linear coil providing optimal homogeneity. The different MRI sequences were parametrized with Paravision PV5 software (Bruker, Wissembourg, France), and acquisition was synchronized to breathing.

Anatomic MRI sequences were used to locate the maternal abdominal aorta and the fetoplacental units. A coronal gradient echo sequence was used first: TR (Repetition Time) 280 m s and TE (Echo Time) 4 m s; matrix 256*256; field of view 8 × 8 cm; section thickness 1.15 mm.