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A longitudinal study of placental perfusion using dynamic contrast enhanced magnetic resonance imaging in murine pregnancy☆

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ABSTRACT

Introduction: To evaluate changes in placental perfusion with advancing gestation in normal murine pregnancy using dynamic contrast enhanced magnetic resonance imaging (DCE-MRI).
Methods: Seven timed-pregnant CD-1 mice underwent DCE-MRI scanning longitudinally on gestational days (GD) 13, 15 and 17. Placentas were segmented into high (HPZ) and low perfusion zones (LPZ) using tissue similarity mapping. Blood perfusion of the respective regions and the whole placenta was quantified using the steepest slope method. The diameter of the maternal central canal (CC) was also measured.
Results: An increase in perfusion was observed between GD13 and GD17 in the overall placenta ($p = 0.04$) and in the HPZ ($p = 0.02$). Although perfusion in the LPZ showed a slight increasing trend, it was not significant ($p = 0.07$). Perfusion, in units of ml/min/100 ml, in the overall placenta and the HPZ was respectively 61.2 ± 31.2 and 106.2 ± 56.3 at GD13 ($n = 19$ placentas); 90.3 ± 43.7 and 139 ± 55.4 at GD15 ($n = 20$); and 104.9 ± 76.1 and 172.2 ± 85.6 at GD17 ($n = 14$). The size of the CC increased with advancing gestation ($p < 0.05$).
Discussion: Using longitudinal DCE-MRI, the gestational age-dependent perfusion change in the normal murine placenta and in its regional compartments was quantified. In mid and late gestations, placental constituent regions differ significantly in their perfusion rates. The CC diameter also showed increase with advancing gestation, which may be playing an important role toward the gestational age-dependent increase in placental perfusion.

1. Introduction

The placenta plays a crucial role in providing adequate nourishment to the developing fetus. It meets the growing metabolic demands of the fetus through appropriate gestation-dependent structural, morphological and functional changes [1]. Increases of the overall size and vascular remodeling, including changes of vessel length, diameter and vessel density per unit volume, in both maternal and fetal circulation compartments are known to occur

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[2–7]. Decrease in vascular resistance is thought to help the efficiency of placental and fetal perfusion [6,8]. Abnormal remodeling of spiral arteries in the placental bed leading to reduced blood supply to the placenta is one of the features observed in obstetric conditions such as preeclampsia and fetal growth restriction (FGR) [9–12]. Indeed, placental ischemia has been implicated as a common factor in serious pregnancy complications — placental abruption, FGR and preeclampsia [13]. Therefore, evaluating placental functional status has become important in obstetric evaluation [14,15], and placental blood perfusion is one of the central indicators of placental function.

Despite its importance as a marker of placental function, our ability to non-invasively measure regional placental perfusion in humans is limited. Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) is a standard technique that uses an exogenous contrast agent (often gadolinium chelate based) for quantifying organ perfusion in ml/min per unit weight or volume of the tissue [16]. Use of MRI contrast agents, however, is contraindicated during pregnancy in humans [17]. In this context, murine models of pregnancy have become an important means for studying and understanding placental development and etiology of various human obstetrical conditions due to the anatomical, functional and cellular similarities between the two [18–23]. Use of contrast enhanced MRI methods in animal models has helped to advance our understanding of the pathophysiology of various conditions [24–26].

DCE-MRI is a powerful method which has been used previously for evaluating placental perfusion in mice [19,27–29]. By this technique, an exogenous MRI contrast agent is injected into the blood stream and, as it traverses the organ of interest, the change in the MRI signal can be observed by taking a series of snapshots of the organ. The temporal contrast-uptake measured from the image time-series provides qualitative and quantitative information about tissue blood perfusion. Previous DCE-MRI studies in mice have shown qualitative differences in placental perfusion between normal and disease conditions [18,30,31] at a given gestation. Although anticipated [32], normal gestational age-dependent change in murine placental perfusion, however, has not been quantified before. While uterine and umbilical flow changes have been characterized across gestation in murine pregnancy [33,34], similar studies measuring regional placental perfusion are scant [35]. Moreover, the placenta is a heterogeneous structure with different constituent regions performing different functional roles. During advancing gestation, the adaptation/changes occurring in these regions may also be distinct. Hence, to understand the normal placental hemodynamic adaptation, it is important to evaluate the regional changes in perfusion as a function of gestational age. In this study, we evaluated the longitudinal changes in murine placental perfusion in mid and late gestation using quantitative analysis of DCE-MRI. Specifically, we evaluated perfusion in the two constituent regions of the murine placenta, the high perfusion zone (HPZ) on the fetal side of the placenta and the low perfusion zone (LPZ) on the maternal side of the placenta, on gestational days (GD) 13, 15 and 17. In previous works, these high and low perfusion zones have been identified in murine placenta using contrast enhanced ultrasound or MRI, based on their characteristic fast or slow uptake of contrast agents [29,36,37]. The HPZ roughly corresponds to the base of the labyrinth zone supplied by the maternal central canal (CC) and the LPZ roughly to the junctional zone. The CC in murine placentas has a functional similarity with that of the spiral arteries in the human placenta in that they both act as important conduits to feed the maternal blood into the region where maternal-fetal metabolic exchange occurs. We also measured the diameter of the maternal CC on the three GDs.

2. Materials and methods

2.1. Animal care and handling

The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Wayne State University (Detroit, MI, USA). Animal care and handling followed the standards set forth by the National Research Council of the National Academies [38]. Timed-pregnant CD-1 mice (n = 7) were obtained from Charles River Laboratories (Wilmington, MA, USA). Pregnancy was confirmed by manual examination on GD12. Mice were kept separately in filter-top rodent cages and fed with *ad libitum* water and food. A regular 12:12 h dark–light cycle and constant temperature (24±1 °C) and humidity (50 ± 5%) were maintained in the animal room, and mice were monitored for food and water intake, vital signs, behavior and activity.

2.2. Magnetic resonance imaging

All MR studies were performed on a dedicated small animal MR scanner 7.0 T, 30 cm bore superconducting magnet (ClinScan, Bruker, Karlsruhe, Germany) interfaced with a Siemens Syngo console. A standard circularly polarized body coil insert was used for imaging. Prior to data acquisition, anesthesia was induced by isoflurane mixed with air to sedate the animals (4% v/v via an induction chamber and then 1.5–2% v/v for maintenance of sedation). Mice were kept under anesthesia throughout the data acquisition. The contrast agent bolus injection was carried out manually over 22 s starting at imaging time point 9. The bolus consisted of 0.5 M gadolinium-based contrast agent, Magnevist (Gadopentetate dimeglumine – Gd-DTPA), diluted 1:10 with saline to 0.25 ml (i.e., 0.0125 mmol of Gd-DTPA), which was injected through a catheter placed in the tail vein. The gadolinium dose was approximately 0.3 mmol/kg for a 42 g pregnant mouse.

The DCE-MRI scan consisted of an initial acquisition for the estimation of baseline tissue longitudinal relaxation time (T_1) followed by the dynamic series acquisition during contrast injection and uptake. A multi-slice two-dimensional (2D) spoiled gradient echo sequence was used for DCE-MRI data acquisition. Imaging parameters were: echo time (TE) = 2.02 ms, repetition time (TR) = 43 ms, resolution = 0.27 × 0.27 × 1.5 mm^3, slice gap = 2 mm, bandwidth = 260 Hz/pixel, and matrix size = 128 × 128. For the estimation of baseline T_1, data was acquired using this sequence with three different flip angles (FA), 5°, 13° and 30° — each with the number of averages (navg) = 8 for high signal-to-noise ratio (SNR). For acquisition during contrast injection and uptake, the same sequence with navg = 1 and FA = 30° was used. A total of 7 slices was acquired with a temporal resolution of 5.5 s, and the data was acquired for 150 time points. During dynamic data acquisition, the contrast agent was injected manually after acquiring 9 volumes which provided a baseline ($S(t{=}0)$) relative to which the contrast-uptake signal ($S(t)$) was observed. The mice were imaged on their respective GDs of 13, 15 and 17 (term gestation 18–21 days). DCE-MRI imaging volumes were placed at the cervix covering the feto-placental units at the end of the right uterine horn. Additionally, volumes were placed in an orientation that allowed for visualization of the midline sagittal section of the placentas.

Tissue contrast agent concentration vs time (C(t)) maps:

The MRI signal obtained using the spoiled gradient echo sequence from a tissue can be expressed as:

$$S = \frac{S_0\left(1 - e^{-\frac{TR}{T_1}}\right)sin\theta}{1 - cos\theta\, e^{-\frac{TR}{T_1}}} e^{-\frac{TE}{T_2}} \quad (1)$$

where T_1 and T_2 are the tissue longitudinal and transverse