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DCE MRI reveals early decreased and later increased placenta perfusion after a stress challenge during pregnancy in a mouse model

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ABSTRACT

Objectives: Stress during pregnancy is known to have negative effects on fetal outcome. The purpose of this exploratory study was to examine placental perfusion alterations after stress challenge during pregnancy in a mouse model.
Material and Methods: Seven Tesla MRI was performed on pregnant mice at embrionic day (ED) 14.5 and 16.5. Twenty dams were exposed to an established acoustic stress challenge model while twenty non-exposed dams served as controls. Placental perfusion was analyzed in dynamic contrast-enhanced (DCE) MRI using the steepest slope model. The two functional placental compartments, the highly vascularized labyrinth and the endocrine junctional zone, were assessed separately.
Results: Statistical analysis revealed decreased perfusion levels in the stress group at ED 14.5 compared to controls in both placenta compartments. On ED 16.5, the perfusion level increased significantly in the stress group while placenta perfusion in controls remained similar or even slightly decreased leading to an overall increased perfusion in the stress group on ED 16.5 compared to controls.
Conclusion: MR imaging allows noninvasive placenta perfusion assessment in this fetal stress mimicking animal model. In this exploratory study, we demonstrated that stress challenge during pregnancy leads to an initial reduction followed by an increase of placenta perfusion.

1. Introduction

Scientific evidence suggests that conditions during pregnancy not only impact fetal development and birth outcome, but also have a profound effect on child and adult health. This concept of developmental origins of health and disease has been a focus of research in recent years [1–3]. Stress during pregnancy has been recognized as one of the risk factors for impaired fetal development [4,5] that may lead to disease programming [6–9]. A measurable outcome of high stress perception during pregnancy is, for example, an increased risk for intrauterine growth restriction (IUGR) [10–13]. The mechanism by which stress mediates this adverse effect is not fully understood, but recent studies suggest that a neuroendocrine stress response may trigger maternal immune and endocrine changes that affect the feto-placental unit [13].

The placenta has a complex vascular net, which supplies the fetus with oxygen and nutrition, that develops and adapts with the course of pregnancy [14]. Disruption of this vulnerable process can lead to alterations in placental blood supply, also referred to as placental insufficiency.

As placental insufficiency is known to be associated with IUGR in humans and mice [13,15], we hypothesize that a stress-induced interference of placental vascular development may mediate the adverse fetal outcome. To investigate this pathway in more detail, we focused this analysis on the role of placental perfusion, which is

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mechanistically related to the placental vascularization, using magnetic resonance imaging (MRI). Dynamic contrast-enhanced (DCE) MRI is a contrast-based, spatio-temporal imaging technique that has been used frequently in the past to analyze placental perfusion in rodents [16–19]. We previously demonstrated that the steepest slope method is a simple and robust method for perfusion quantification of the mouse placenta using DCE MRI datasets [20]. Furthermore, we have recently shown that the placenta consists of two perfusion territories, which should be analyzed separately as simple averaging across the whole placenta may mask important differences between groups if only one of the two compartments shows pathologic signs, e.g. resulting from stress during pregnancy [6]. The aim of this study was to apply this method of quantitative analysis of blood flow to investigate the effect of stress during pregnancy on placenta perfusion. This work should be viewed as an exploratory hypothesis-generating study that is supposed to support the design of fully-powered future research studies for more in-depth analyses of the causal links between stress and perfusion alterations.

2. Material and Methods

2.1. Mice

Female BALB/c and male DBA/2J mice were purchased at the age of 8 weeks from Charles River (Germany). Mice were kept in an animal facility with 12 h light/dark cycle. Food and water were provided *ad libitum*. A timed mating was performed. The detection of a vaginal plug the following day at noon was considered to be embryonic day (ED) 0.5. To test the effect of prenatal stress exposure on placenta perfusion, one group of pregnant DBA/2J-mated BALB/c mice (n = 20) was exposed to an established sound challenge model, previously described in detail [13]. In brief, mice were exposed to a sound challenge, generated by a rodent repellent device (Conrad Electronics) on ED 12.5 and 14.5. A second group of pregnant mice served as controls (n = 20) without exposure to a sound challenge. 10 mice of each group were examined with MRI on ED 14.5 after sound challenge. The remaining 10 mice per group were scanned on ED 16.5. All animal care and experimental procedures were conducted according to institutional guidelines and conformed to requirements of the German Animal Welfare Act. Ethical approvals were obtained from the State Authority of Hamburg (Germany).

2.2. Magnetic resonance imaging

MRI was performed on pregnant dams on ED 14.5 (stressed n = 10; control n = 10) and 16.5 (stressed n = 10; control n = 10) using a dedicated small-animal 7T MR-scanner (Clinscan, Bruker, Germany) and a circularly polarized transmit/receive coil with an inner diameter and resonator length of 40 mm. An isoflurane/O_2 inhalation mixture (1–1.5% vol/vol) was used for anaesthetization during data acquisition. The respiration rate was closely monitored and maintained at 70–85 breaths/min. After MRI was performed, dams were sacrificed.

In a first step, a high-resolution turbo-spin-echo (TSE) imaging sequence was acquired in coronal orientation to locate the foetuses and the placentas required for correctly focussing the perfusion MRI scan on the anatomical area of interest. This TSE sequence was acquired with repetition time (TR) = 3.1 s, echo time (TE) = 64 ms, field-of-view (FoV) = $35 \times 50\ mm^2$, flip angle = 180°, matrix = 448×640, and 16 slices with 4 mm thickness.

Next, a coronal dual-echo three-dimensional T1-weighted gradient-echo sequence was used to investigate the perfusion in the placenta (TR: 10 ms, TE: 1.78/4 ms, FoV: 40 mm^2, flip angle: 20°, matrix: 128×128, slice thickness 1 mm, slices: 16). After acquisition of four baseline image volumes, 100 μl of gadobenate dimeglumine (Multihance, Bracco, Germany) with a dose of 0.16 mmol/kg body weight diluted 1:10 in saline was injected via a tail vein catheter, followed by a 100 μl saline bolus. After injection of contrast agent, a total of 50 three-dimensional DCE MR images with a temporal resolution of 10 s were acquired (~8.3 min total scan time).

2.3. Perfusion analysis

DCE-MRI does not measure the perfusion directly but only the contrast agent dynamics. Thus, further calculations are required to quantify the perfusion based on the measured concentration time curves. Within this context, the steepest slope model is a relatively simple and robust computational technique to calculate perfusion values from perfusion-weighted MRI datasets [20].

Every placenta suitable for perfusion analysis in terms of full coverage in the image sequence was manually segmented in each dam as previously described [20]. After a baseline correction of the DCE-MRI data, consisting of a voxel-wise subtraction of the average signal intensity from the first four time points from every time point, perfusion analysis was performed using the steepest slope model as introduced by Miles et al. [1], as defined by:

$$F = \frac{\max(C'(t))}{\max(AIF(t))}$$

where $C'(t)$ denotes the first derivative of the concentration time curve $C(t)$, which was calculated using piecewise linear regression, and $AIF(t)$ the arterial input function. The AIF was manually selected in the maternal kidney hilus. To increase the accuracy of perfusion quantification and reduce noise effects in the discrete concentration time curve measurements, an adapted gamma variate function as described by Johnson et al. [4] was fitted to each concentration time curve to prior to steepest slope model analysis.

It was recently shown that the placenta consists of a high and low perfusion zone that should be analyzed separately to achieve a higher sensitivity for detecting subtle perfusion differences [6]. For this reason, each segmented placenta was automatically divided into two perfusion compartments using a k-means clustering algorithm based on bolus arrival time [2,3,6]. The application of this algorithm results in one compartment, represented by early bolus arrival times, corresponding to the placental base and the central labyrinth zone, where maternal blood arrives with high velocity through central arterial canals and flows into the sinusoids of the labyrinth (high flow zone). The second compartment, with prolonged bolus arrival times, mirrors blood simmering through the peripheral labyrinth and venous backflow through the junctional zone and decidua (low flow zone). As maternal arterial blood flow to the placenta is known to be a valuable marker in pregnancy complications such as IUGR, we primarily focus on perfusion in the high flow zone in this work as it can be expected to be highly correlated with the arterial inflow. However, to investigate the importance of compartment differentiation for perfusion analysis of the placenta, we also analyzed perfusion in the low flow compartment and the perfusion averaged over the entire placenta covering high and low flow zone (Fig. 1).

2.4. Statistical analysis

A paired *t*-test was used to test for significant differences between perfusion values of the different groups using Matlab (The Mathworks, Natick, USA). All data are presented as mean ± standard deviation (SD). A probability of $p < 0.05$ was considered to indicate statistical significance.