Placenta 34 (2013) 899–906

Contents lists available at SciVerse ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Application of the steepest slope model reveals different perfusion territories within the mouse placenta

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ARTICLE INFO

Article history:
Accepted 18 June 2013

Keywords:
Placental perfusion
Steepest slope model
Dynamic contrast-enhanced MRI

ABSTRACT

Objectives: The steepest slope model is a numerically robust and fast method for perfusion quantification. The purpose of this study was to evaluate if the steepest slope model can be used for quantifying placental perfusion in mice based on dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) datasets.
Material and methods: T1-weighted DCE MRI was performed in 5 pregnant BALB/c mice on gestation day (gd) 14.5 and in 5 mice on gd 16.5 using a 7T small animal MRI scanner. The placentas were manually delineated in the DCE datasets and the arterial input function (AIF) was selected from the kidney hilus. Placental perfusion was determined on a voxel-by-voxel basis using the steepest slope model. Perfusion was averaged over the entire placenta as well as separately calculated for the high-flow compartment within the central labyrinth zone and for the remaining low-flow placenta tissue. The AIF selection was independently performed by two observers for assessment of inter-observer differences.
Results: Mean perfusion on gd 14.5 was 135 ml/min/100 ml (standard deviation SD: 29 ml/min/100 ml placenta) and 112 ml/min/100 ml on gd 16.5 for the whole placenta (SD: 32 ml/min/100 ml). Perfusion in the high flow compartment in the central labyrinth was significantly higher ($p \leq 0.002$) than in the low-flow compartment including the remaining placenta tissue: 184 ml/min/100 ml (SD: 39 ml/min/100 ml) vs. 119 ml/min/100 ml (SD 28 ml/min/100 ml) on gd 14.5 and 158 ml/min/100 ml (SD: 58 ml/min/100 ml) vs. 114 ml/min/100 ml (SD: 52 ml/min/100 ml of placenta) on gd 16.5. The mean relative inter-rater observer difference was 6%.
Conclusion: The steepest slope model is a computationally simple method, which allows perfusion quantification in the mouse placenta. Furthermore, the results of this work indicate that the different placental compartments should be analyzed separately to prevent biased results due to averaging.

1. Introduction

The mouse placenta, like the human placenta, consist of three compartments or layers [1]: The outer maternal layer comprises the uterine decidua, as well as maternal feeding vessels [2], the junctional zone, which consists of fetoplacental trophoblast cells, and [3] the labyrinth, where the feto-maternal exchange occurs. Maternal blood enters the implantation site through uterine arterial vessels and flows into the spiral arteries. The spiral arteries merge into fewer centrally located arterial canals at the level of the trophoblast giant cell layer that carry the maternal blood to the fetal side of the placenta. The maternal blood then flows back to the maternal side through the intervillous spaces of the labyrinth, in a countercurrent direction to the fetal capillary blood flow [4].

Placental perfusion alterations have been associated with gestational pathologies in mice, humans and other mammalian species [1–3]. Placental perfusion is routinely determined in the

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0143-4004/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.placenta.2013.06.304

clinical setting by assessing blood flow changes in the preplacental (uterine) or postplacental (umbilical, fetal) vasculature using Doppler ultrasound to predict pregnancy outcome [5–7]. Nevertheless, early identification of women at risk for intrauterine growth restriction (IUGR) or preeclampsia remains difficult, especially in low-risk cohorts [8]. It has been hypothesized that perfusion in the placenta could be used as a sensitive biomarker for negative changes, even in an early stage [9]. However, Doppler ultrasound can only estimate placental perfusion indirectly by measuring the impedance of flow in the uterine artery [6], while direct perfusion quantification using 3D power Doppler ultrasound has been proven to be unreliable [10]. Within this context, dynamic contrast-enhanced magnetic resonance imaging (DCE MRI) has gained increased attraction lately for analyzing placental perfusion in mice [11,12]. Even though the administration of contrast agent is not permitted in human pregnancy, DCE MRI of the mouse placenta offers highly valuable information for basic understanding of disease mechanisms, which may be transferable to humans with alternative techniques that do not require an application of contrast agent such as arterial spin labeling MRI. DCE MRI allows a time-resolved acquisition of 3D datasets, which display the passage of previously injected contrast agent through the body and different tissues. Thus, an analysis of the placenta perfusion in terms of estimating perfusion parameters from the corresponding indicator dilution curves within a volume-of-interest becomes possible using this imaging technique. Pharmacokinetic compartment models [11,12] or semi-quantitative model-free approaches [13] have been applied in previous placenta perfusion analysis studies. However, a reliable perfusion analysis using compartment models requires a DCE MRI acquisition with high temporal resolution. Therefore, only fast 2D image acquisition techniques can be used and the resulting datasets usually exhibit a rather low spatial resolution, which leads to the problem that it may not be possible to differentiate important anatomical details within the placenta.

Compared to this, the outcome of semi-quantitative model-free analyses may be highly affected by the imaging parameters and contrast agent injection protocol used. Furthermore, model-free approaches may also exhibit considerable observer dependencies. Therefore, study results from different centers may not be comparable to a sufficient extent.

The steepest slope model, which was originally presented by Miles [14], who adapted an established nuclear medicine technique to dynamic computed tomography datasets, is known to be numerically robust and fast while also enabling a quantitative perfusion analysis.

The aim of this study was to evaluate if the steepest slope model can be applied for a quantification of the perfusion in the mouse placenta based on DCE MRI datasets. Furthermore, a secondary aim of this study was to evaluate the possibility of analyzing the perfusion separately for different perfusion compartments within the placenta.

2. Methods

2.1. Animals

For experiments, female BALB/c mice were mated with male DBA/2J mice (age: 8–10 weeks) at our institution. The detection of a vaginal plug the following day at noon was considered to be gestation day (gd) 0.5. Imaging was performed on gd 14.5 in five mice and on gd 16.5 in five different mice. Mice were sacrificed after imaging. All studies were approved by the local animal protective committee.

2.2. Magnetic resonance imaging

Magnetic resonance imaging was performed using a dedicated small animal MR scanner at 7.0T (ClinScan, Bruker BioSpin, Ettlingen, Germany) using a circularly polarized transmit/receive-coil with an inner diameter of 40 mm and a resonator length of 40 mm.

Mice were anesthetized with isoflurane and maintained on an isoflurane/O_2 inhalation mixture (1–1.5% vol/vol) throughout data acquisition, with the respiration rate maintained between 70 and 85 breaths/min. A catheter was placed in the tail vein and tested for intravascular position using a 100 μl saline bolus (Syringe size: 1 ml, catheter length: 70 cm; catheter diameter: 0.28 mm, dead volume from needle to tip: 43 μl, needle size: 30G). Cyanoacrylate was used for catheter fixation. Anesthetized mice were placed in prone position with the abdomen at the center of the coil. The animals were covered with a water-heated pad to retain body temperature.

A dual-echo turbo-spin-echo (TSE) MR imaging sequence (TR: 3100 ms; TE: 14/64 ms; FoV: 50 mm, flip angle: 180°, slice thickness 0.8 mm) in coronal orientation was used to locate the placentas and the fetal mice (Fig. 1). For dynamic MR imaging, a three-dimensional T1-weighted gradient-echo sequence in coronal orientation was used (TR: 10 ms, TE: 1.78 ms, FoV: 40 mm, flip angle: 20°, matrix: 128, slice thickness 1 mm, slices: 16, repeat measurements: 50, temporal resolution per 3D volume: 10 s).

After acquisition of four baseline 3D image volumes, the contrast agent followed by a saline flush was injected via the tail vein catheter. For this purpose, the commercially available gadolinium chelate (Multihance, Bracco, Germany), which was diluted 1:10 with saline prior to injection, was used as contrast agent. Injections via the tail vein catheter were performed manually by a single investigator (CCM; 4 years of dedicated experience in animal research) during acquisition of the fifth 3D image volume. The 100 μl bolus of the diluted Gadolinium solution was injected in approximately 4 s and followed by a 100 μl saline flush, which was also injected in 4 s after changing the syringe, which took about 2 s. The Gadolinium dose was approximately 0.16 mmol/kg for a 30 g pregnant mouse. This relatively low dose was

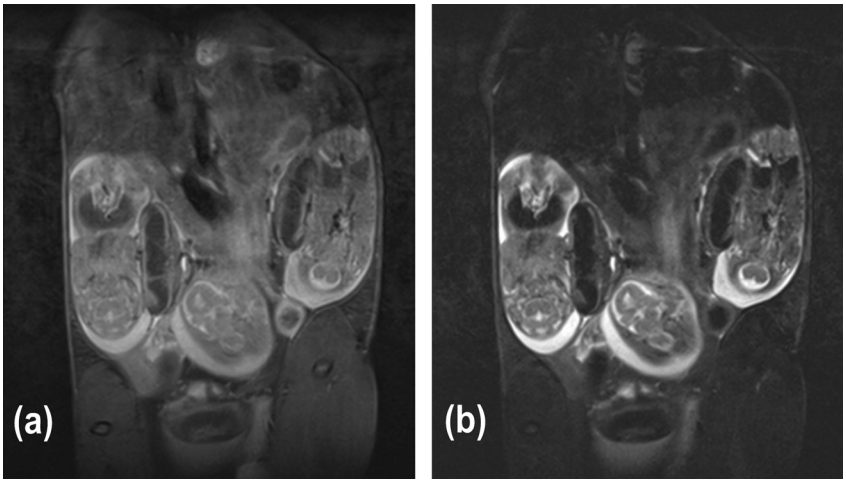

Fig. 1. Dual-echo turbo-spin-echo (TSE) MR imaging sequences in coronal orientation of the maternal abdomen (gestation day 16.5). Three mouse fetuses and two corresponding placentas can be observed in this image The PD-weighted (TE: 64) sequence (a) gives more refined contrast for visualization of placental compartments, while the T2-weighted sequence (b) allows easier fetal assessment.