Placenta xxx (2017) 1–8

Contents lists available at ScienceDirect

Placenta

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

BOLD-MRI demonstrates acute placental and fetal organ hypoperfusion with fetal brain sparing during hypercapnia

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ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form
4 September 2017
Accepted 8 September 2017

Keywords:
Placental circulation
Pregnancy
Hypercapnia
fMRI
Ultrasonography
Doppler
Animal experimentation
Pre-eclampsia

ABSTRACT

Introduction: We evaluated changes in placental and fetal hemodynamics in rodents during acute hypercapnia using BOLD-MRI and Doppler ultrasound.
Methods: Animals were anesthetized with pentobarbital and, in consecutive 4-min periods, breathed: air, 21%O2:5%CO2, and 95%O2:5%CO2. **BOLD-MRI**: Pregnant ICR mice (n = 6; E17.5) were scanned in a 4.7-T Bruker Biospec spectrometer. Placenta and fetal liver, heart and brain were identified on True-FISP images. Percent change in signal intensity (SI) were analyzed every 30 s from T2*-weighted GE images (TR/TE = 147/10 ms). **Doppler:** Pregnant Wistar rats (n = 6; E18-20) were anesthetized with pentobarbital and received abdominal Doppler ultrasound. Umbilical artery pulsatility index (PI) and fetal heart rate were assessed at baseline and after two minutes of both hypercapnic challenges.
Results: **BOLD-MRI**: Normoxic-hypercapnia caused immediate marked reduction in SI in placenta (−44% ± 5.5; $p < 0.001$), fetal liver (−32% ± 6.4; $p < 0.001$) and fetal heart (−53% ± 9.9; $p < 0.001$) but only minor changes in fetal brain (−13% ± 3.4; $p < 0.01$), suggesting fetal brain sparing. **Doppler:** Normoxic-hypercapnia caused a marked increase in umbilical artery PI (+27.4% ± 7.2; $p < 0.001$) and a reduction in fetal heart rate (−48 bpm; 95%CI -9.3 to −87.0; $p = 0.02$), suggesting acute fetal asphyxia.
Conclusions: Brief maternal hypercapnic challenge caused BOLD-MRI changes consistent with acute placental and fetal hypoperfusion with fetal brain sparing. The same challenge caused increased umbilical artery PI and fetal bradycardia on Doppler ultrasound, suggestive for acute fetal asphyxia. BOLD-MRI may be a suitable noninvasive imaging strategy to assess placental and fetal organ hemodynamics.

1. Introduction

The fetal circulatory changes that follow acute asphyxia are not fully understood. Most available data comes from Doppler ultrasound studies in chronically asphyxiated pregnancies in sheep [1], non-human primates [2] and humans [3], which suggest the shunting of oxygenated blood from the umbilical vein to the fetal brain through the ductus venosus at the expense of other fetal organs [1–3], a phenomenon called brain sparing or centralization. However, Doppler ultrasound cannot assess maternal, placental and fetal vessels simultaneously, and cannot make repeated

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https://doi.org/10.1016/j.placenta.2017.09.005
0143-4004/

measurements at short fixed time intervals, therefore it is a limited tool when evaluating real-time acute fetal hemodynamic responses.

Magnetic resonance imaging (MRI) has been used to study the placenta. Although dynamic-contrast MRI with gadolinium has been used to assess utero-placental blood flow (UPBF) in response to chronic hypoxia in pregnant mice [4], the use of gadolinium is limited by slow placental transfer, by the large doses required for fetal imaging and by the potential for nephrogenic systemic fibrosis in humans [5]. On the other hand, functional imaging based on blood oxygen level dependent (BOLD) MRI has been used to infer changes in perfusion without the need for contrast administration. BOLD-MRI exploits the interference of paramagnetic deoxyhemoglobin on the external magnetic field, which reduces signal intensity (SI), while diamagnetic oxyhemoglobin has the reverse effect [6]. BOLD-MRI SI is measured in arbitrary units, which limits the assessment of absolute perfusion or oxygenation, however proportional change can be evaluated to assess both regional differences and the dynamic response to a physiological challenge [7]. Studies in animals and humans have demonstrated that changes in BOLD-MRI SI indicate changes in regional brain perfusion [8]. BOLD-MRI has also been used to image changes in regional intrauterine fetal organ perfusion following hypoxia in pregnant sheep [9,10] and following hyperoxia in pregnant humans [11,12]. Our group recently used BOLD-MRI in non-pregnant rodents during a brief normoxic hypercapnic challenge, part of a protocol termed hemodynamic response imaging (HRI), and reported a reduction in hepatic SI [13–15].

Based on these studies, we decided to evaluate HRI as a novel non-invasive imaging strategy of uteroplacental and fetal hemodynamics. SI is dependent upon the following: 1) blood volume and arterial-venous admixture in the target organ, 2) cardiac output and regional perfusion, 3) oxygen carrying capacity (hemoglobin concentration and oxygen saturation, the latter in turn dependent upon PaO_2, 4) the oxy-hemoglobin dissociation curve, itself dependent on the balance between left-shifting factors (fetal vs adult hemoglobin) and right-shifting factors ($PaCO_2$, 2,3DPG, and acidosis) and 5) oxygen extraction. In view of the above, there are some potentially limiting factors that may weaken the assumption that changes in SI reflect changes in perfusion, especially during CO_2 challenge. Most studies that have assessed the effect of hypercapnia on the BOLD SI have used hyperoxic hypercapnia (with carbogen, 5% CO_2 with 95%O_2), where the predominant effect on SI is due to increased oxygen saturation. By contrast, normoxic hypercapnia (5%CO_2 in 21%O_2) would be expected to shift the oxyhemoglobin dissociation curve to the right (reducing SI). Typically, regional changes in SI for different fetal organs do imply regional changes in fetal organ perfusion (or regional changes in oxygen extraction). Nevertheless, BOLD SI is not a direct measure of perfusion and uncertainty about whether BOLD changes in the placenta correspond to oxygen saturation changes in the fetal or maternal blood makes the interpretation of signal changes in this organ uncertain. In view of these theoretical considerations, it was important to correlate our findings with Doppler ultrasound pulsatility, the most widely used surrogate measure for blood flow measurement in current clinical practice, which actually measures vascular impedance rather than blood flow. In particular we were interested to assess whether, during normoxic hypercapnia, the reduction in BOLD-SI for placenta and fetal liver was accompanied by increased umbilical artery Doppler pulsatility index (PI) and by fetal bradycardia.

We hypothesized that normoxic hypercapnia may reduce the SI of the placenta and major fetal organs (fetal liver and fetal heart) but with relative fetal brain sparing (minimal change in fetal brain and an increase in the fetal brain:liver ratio).

2. Methods

2.1. Ethics

Animal experiments were performed in accordance with the guidelines and approval of the Animal Care and Use Committee (IACUC) of the Hebrew University which holds NIH approval (OPRR-A01-5011). Animals in this study were adult female ICR mice or Wistar rats with timed pregnancy and were anesthetized with 80 mg/kg intraperitoneal pentobarbital (CTS group, Hod-Hasharon, Israel). Following the study, animals were euthanized by a lethal dose of intraperitoneal pentobarbital.

2.2. CO_2 challenge

For both the BOLD-MRI and the Doppler ultrasound studies, all animals were placed supine and were spontaneously breathing. Animals were attached by a loose-fitting facemask to a breathing system with 4 l/min fresh gas flow; for three consecutive 4 min periods, animals breathed the following gases in sequence: medical air (21% O_2, 79% N_2), air-carbon dioxide (5% CO_2, 21% O_2, 74% N_2) and carbogen (5% CO_2, 95% O_2); all gases were supplied by Oxygen & Argon Works Ltd., Israel. Respiratory safety monitoring in the MRI scanner was verified by an MRI-compatible chest wall pneumotachometer (SA instruments, Inc. Stony Brook, NY).

2.3. Hemodynamic response imaging (HRI)

HRI, using BOLD-MRI, was performed in adult female ICR mice with timed pregnancy (n = 6; E18) using a 4.7-T Bruker Biospec spectrometer (Bruker Medical, Ettlingen, Germany) with a birdcage coil (3 cm diameter). Fast coronal True-FISP (fast imaging with steady state precession) images (repetition time/echo time = 3/1.5 ms) of the abdomen region were initially performed to locate fetuses and organs of interest. Changes in placental and fetal perfusion were analyzed from coronal T_2^*-weighted gradient echo images (repetition time/echo time = 147/10 ms; field of view = 5.4 cm^2; in-plane resolution = 210 μm; slice thickness = 1 mm; 2 averages; 256*256 pixels; resulting in a temporal resolution of 37 s) as previously described [13–15]. A total of 22 dynamic repetitions were recorded. Different regions of interest (maternal liver, placenta, fetal heart, fetal liver and fetal brain) were identified manually on the True-FISP images using a program developed in our laboratory based on IDL software (Interactive Data Language, ITT Visual Information Solutions, Boulder, Colorado). Fetal and placental HRI data were averaged for each dam from at least 5 pups. The percentage change in SI (ΔS), induced by normoxic-hypercapnia (ΔS_{CO2}) or hyperoxic-hypercapnia (ΔS_{O2}) was calculated from the average of six repetitions for each condition, while the first two repetitions were ignored. The calculations included only pixels with statistical threshold of $P < 0.05$ as previously described [16]. We assessed the within-subject and between-subject variation coefficient in SI at baseline.

2.4. Doppler ultrasound

We performed Doppler ultrasound in adult female Wistar rats with timed pregnancy (n = 6; E18-20). Doppler ultrasound indices of vascular resistance in the umbilical artery and ductus venosus were assessed. Abdominal scan was performed using a transvaginal 5–9 L probe, 5.0–9.0 MHz, GE Voluson E6 (GE Medical Systems, Milwaukee, WI, USA).

The umbilical artery was assessed by transabdominal ultrasound using the following criteria: (1) the magnification of the

Please cite this article in press as: Y. Ginosar, et al., BOLD-MRI demonstrates acute placental and fetal organ hypoperfusion with fetal brain sparing during hypercapnia, Placenta (2017), https://doi.org/10.1016/j.placenta.2017.09.005