Analysis of Angiogenesis in Mouse Pregnancy Using BSA-Gd-DTPA Enhanced 3D-MRI

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Introduction:

The development of a complex maternal-fetal vascular network to support the fetal growing demands for oxygen and metabolites is essential for both implantation and placentation[1],[2]. Embryo implantation failure is clinically relevant to recurrent pregnancy loss, low success of *in vitro* fertilization and diseases of pregnancy like preeclampsia[3]. Intrauterine growth restriction and preeclampsia associated with placental vasculopathy and poor obstetric outcomes are directly linked to abnormal placental vascular development[1]. Implantation that occurs in the mouse at E4-4.5 (vaginal plug=E0.5), is governed by decidualization associated with uterine vascular permeability and angiogenesis in order to supply the fetal needs until the mature placenta is formed (~E10). Macromolecular contrast enhanced MRI was applied here to study vascular development and angiogenesis in maternal-fetal interactions during pregnancy.

Materials and Methods:

Animal model: Pregnant female ICR mice (12 weeks) were scanned by MRI at E5, 6, 13, 17.

Contrast agents: Biotin-BSA-GdDTPA and BSA-fluorescein/rhodamine were prepared as reported[4]. Evans blue (EB) was used as reported[5].

MRI experiments: Animals were placed in a 4.7T horizontal Bruker (Germany) Biospec spectrometer and a whole body excitation coil was used. 3D-GE: a series of images with 15, 5, 30, 50, 70 degrees flip angles were acquired to determine the precontrast R1 (TR 10ms, TE 3.6ms, 2 averages, spectral width 50000Hz, matrix 128x128x64 (zero filled to 128), FOV 5x5x5 cm). For dynamic postcontarst imaging, images were obtained with a 15 degrees flip angle and animals were followed for 30min after intravenous administration of MR contrast agent (MR-CA) via the tail vein. Immediately after MRI, animals were i.v. administered with EB (15min) and with BSA-rhodamine (5min) and then sacrificed. Uteri were processed for histology.

MRI data analysis: Signal intensities (SIs) were analyzed using pre- and postcontrast 3D-GE-mean signal intensities at ROIs of implantation sites (E5 and 6) or placentas (E13 and 17) and mean SIs and MR-CA concentrations were obtained. Changes in CA concentrations were used for derivation of APS (apparent permeability surface area product) and fPV (plasma volume fraction)[6].

Histology: Uteri including implantation sites (E5 and 6) or embryos and placentas (E13 and 17) were excised, fixed, embedded in paraffin and sectioned (4μm) and stained for biotin-BSA-GdDTPA with avidin-FITC. Smooth muscle actin was also immunostained[6].

Results:

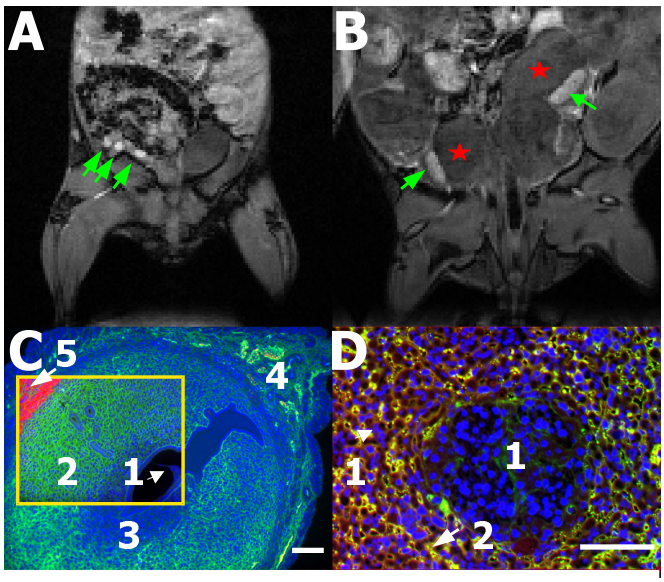
The MR-CA was detected in the vasculature and in the placenta (E13, 17) immediately after intravenous administration (~3min). The detection of MR-CA in implantation sites (E 5, 6) was delayed to ~6-9min after administration. Signal enhancement was specific to implantation sites (Fig 1A) at early pregnancy and to placenta (Fig 1B) at late pregnancy, with no enhancement in the embryo or other parts of the uterus. Verification of pregnancy immediately postimplantation was completed using intravenous administration of EB and the appearance of "bluing reaction" at the implanted sites[5]. While fPV increased as the pregnancy progresses, permeability (APS) was only apparent at early pregnancy (E5 and 6). At later stages (E13, 17), no permeability was detected. This was further confirmed by fluorescent staining of the MR-CA: although MR-CA accumulation was not apparent in the embryo at any pregnancy stage, MR-CA was detected in the stroma at the implantation site with decreasing gradient towards the decidua surrounding the embryo (Fig 1C). At later stages, MR-CA was restricted to vessels carrying maternal blood at the labyrinth of the mature placenta (Fig 1D).

Discussion:

Analysis of MRI and fluorescent data revealed permeability in the stroma of the subdecidual area at implantation sites (E5 and 6) but not in mature placentas (E13 and 17), although MR-CA was detected in placental maternal blood vessels (but not in fetal vessels). We speculate that immediately postimplantation, in the absence of placenta, permeability serves to supply the growing embryo with gases and nutrients and probably enables provisional matrix remodeling and consequent angiogenesis. Following completion of placentation, the fetus is provided by mature placental vasculature (via diffusion and active transport between maternal and fetal circulations). MRI follow-up of angiogenic processes associated with implantation and placentation can further be implicated to study associated pathological conditions as preeclampsia.

Fig 1.

Biotin-BSA-GdDTPA enhanced implantation sites of mouse embryo (arrows) at E5 (A) and placental labyrinths (arrows) next to fetuses in amniotic sacs (stars) at E17 (B). Cross section (C) of an implantation site in A: embryo (1), biotin-BSA-GdDTPA (green) extravasated in stroma of subdecidual area (2), decidua (3) and early administered biotin-BSA-GdDTPA in blood vessels (green) co-localized with late administered BSA-rhodamine (red/yellow) are indicated (4). On a separate section (yellow frame), smooth muscle actin stained myometrium and mature blood vessels (5). Cross section (D) of placental labyrinth in B: nucleated RBCs within fetal circulation (1), early administered biotin-BSA-GdDTPA (green) co-localized with late administered BSA-rhodamine (red/yellow) indicating non-permeable maternal blood vessels (2). Scale bar 100μm.



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