Placenta 58 (2017) 46–51

Contents lists available at ScienceDirect

Placenta

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

Arterio-venous fetoplacental vascular geometry and hemodynamics in the mouse placenta

Monique Y. Rennie [a], Lindsay S. Cahill [a], S. Lee Adamson [b, c, d], John G. Sled [a, b, d, e, *]

[a] Mouse Imaging Centre, The Hospital for Sick Children, Toronto, Ontario, Canada
[b] Translational Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada
[c] Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada
[d] Department of Obstetrics and Gynecology, University of Toronto, Toronto, Ontario Canada
[e] Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

ARTICLE INFO

Article history:
Received 30 May 2017
Received in revised form
8 August 2017
Accepted 11 August 2017

Keywords:
Arteries
Fetoplacental vasculature
Hemodynamics
Micro-computed tomography
Mouse
Veins

ABSTRACT

Introduction: The fetoplacental vasculature network is essential for the exchange of nutrients, gases and wastes with the maternal circulation and for normal fetal development. The present study quantitatively compares arterial and venous morphological and functional differences in the mouse fetoplacental vascular network.
Methods: High resolution X-ray micro-computed tomography was used to visualize the 3D geometry of the arterial and venous fetoplacental vasculature in embryonic day 15.5 CD-1 mice (n = 5). Automated image analysis was used to measure the vascular geometry of the approximately 4100 arterial segments and 3200 venous segments per specimen to simulate blood flow through these networks.
Results: Both the arterial and venous trees demonstrated a hierarchical branching structure with 8 or 9 (arterial) or 8 (venous) orders. The venous tree was smaller in volume and overall dimensions than the arterial tree. Venous vessel diameters increased more rapidly than arteries with each successive order, leading to lower overall resistance, although the umbilical vein was notably smaller and of higher resistance than these scaling relationships would predict. Simulation of blood flow for these vascular networks showed that 57% of total resistance resides in the umbilical artery and arterial tree, 17% in the capillary bed, and 26% in the venous tree and umbilical vein.
Discussion: A detailed examination of the mouse fetoplacental arterial and venous tree revealed features, such as the distribution of resistance and the dimension of the venous tree, that were both morphologically distinct from other vascular beds and that appeared adapted to the specialized requirements of sustaining a fetus.

1. Introduction

In the adult circulation, arteries and veins differ in both function and structure. Smooth muscle in the walls of arteries mediate autoregulation of blood flow and are organized in a manner that provides for a large pressure drop and low transit time between the major conduit vessels and the capillary bed. Veins by comparison are highly compliant with little or no contractile function, present low hemodynamic resistance and operate under low blood pressure conditions. The placenta has a number of differences from adult organs both in how it functions and how it is constructed that could provide insight into its unique architecture. In humans, the placenta is hemochorial [1] with two distinct (fetal and maternal) circulations. Exchange of oxygen and nutrients occurs between fetoplacental capillaries within the placental villi and maternal blood within the intervillous space. From a hemodynamic perspective, an important functional requirement is that the fetoplacental capillaries maintain adequate internal pressure to avoid being collapsed by the maternal blood pressure [2]. Another hemodynamic consideration is that the fetoplacental vasculature is separated from the fetal circulation by the long conduit vessels of the umbilical cord, typically 50–60 cm long in humans [3], and presenting a non-negligible hemodynamic resistance [4].

Abbreviations: CT, computed tomography; E, embryonic day.

* Corresponding author. Hospital for Sick Children, Mouse Imaging Centre, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada.
E-mail address: john.sled@utoronto.ca (J.G. Sled).

http://dx.doi.org/10.1016/j.placenta.2017.08.007
0143-4004/

Functionally, it is not clear that the fetoplacental vessels autoregulate blood flow. Another unique aspect of the placenta is that it develops and matures rapidly to keep pace with the requirements of the growing fetus. The arterio-venous oxygen gradient in the fetoplacental circulation is also reversed with respect to other organs aside from the lung. In the placenta, deoxygenated blood enters from the arteries, receives oxygen from the maternal circulation via the fetoplacental capillaries, and delivers oxygen to the fetus via the umbilical vein. These differences from other organs motivate a closer examination of how arterial and venous vessels are organized in the placenta. While the organization and hemodynamic characteristics for the fetoplacental arteries have been described, less is known about the overall network structure that includes the venous vessels.

3D imaging of the placenta using X-ray micro-computed tomography (micro-CT) has been used by a number of groups to investigate the structure of the fetoplacental arterial tree [5–7]. Venous structure, while also amenable to 3D imaging [8], has been largely unstudied. The considerations of resolution, placental size, and vessel dimensions as well as the practical aspects of obtaining samples under highly controlled experimental conditions make this technology particularly amenable to examining the mouse placenta. Moreover, computational analysis methods enable detailed measurement and hemodynamic modelling based on these 3D images [9,10]. While the mouse and human placenta differ, most importantly in size and also the organization of the human placenta into subunits (cotyledons), there are important similarities [11,12]. Specifically, the mouse placenta is hemochorial and shares many common structural characteristics, cell types, genetic pathways, and functions with humans [1]. Here we present a structural and hemodynamic analysis of the fetoplacental vasculature in the mouse with the goal of understanding the structure of this vascular bed in light of its unique functional requirements.

2. Materials and methods

2.1. Mice

Experimental procedures were approved by the Animal Care Committee of the Toronto Centre for Phenogenomics and conducted in accordance with guidelines established by the Canadian Council on Animal Care. CD-1 mice were purchased from Charles River Laboratories (Montreal, QC, Canada). Males were mated in-house with virgin females aged 8–14 weeks and the morning that a vaginal copulation plug was detected was designated embryonic day (E) 0.5. All dams were euthanized at E15.5. This gestational age was chosen based on the relative ease of sample preparation; there is a higher rate of perfusion failure at E13.5 because of fragile vessels and after E17.5 the vessels are prone to vasospasm [8].

2.2. Injection of contrast agent

Detailed methods for preparing the fetoplacental vasculature for micro-CT imaging have previously been described [13,14]. E15.5 conceptuses were surgically exposed and a double lumen cannula [13] was inserted into either the umbilical artery for arterial only perfusions or into the umbilical vein for venous only perfusions. The alternate vessel was nicked to serve as a vent. Blood was cleared from the vasculature using heparinized saline containing xylocaine [13]. Contrast agent (MV-122 Microfil, Flow Tech Inc., Carver, MA, USA) was then manually infused until it was seen entering the capillary bed. The umbilical vessels were ligated to maintain pressure during polymerization, after which placentas were immersed in formalin and later mounted in agar.

2.3. Micro-CT imaging and vascular segmentation

3D datasets were acquired for fetoplacental arterial ($n = 5$) and venous ($n = 5$) contrast-enhanced specimens with the surrounding tissue still intact using a Skyscan 1272 micro-CT scanner (Skyscan, Belgium). With the X-ray source at 50 kV and 201 μA, the specimen was rotated 360° in 0.4° increments, generating 900 views in ~2 h which were reconstructed into data blocks with a 13.4 μm voxel size. Vascular surface renderings were generated from micro-CT data to visualize the arterial or venous vasculature [8].

The arterial or venous vasculature was automatically segmented, identifying vessel segments and bifurcations using an algorithm as previously described [15]. The algorithm returned the centre lines of a connected vessel tree and a tubular model for which the lengths, diameters, and connectivity of each vessel segment were described. Measurements of vessel segment numbers, diameter scaling coefficient, and the distribution of vessel diameters were extracted from the resultant tubular models for all vessels >35 μm [10,15,16]. Branching topology was assessed via Strahler ordering, a widely used method for designating levels of a tree that is based on how blood vessels connect [16]. In brief, terminal vessel segments were labeled as order one and then parent vessel segments were assigned either: (1) the greater of the two orders for the daughter branches or (2) the order of the daughter plus one where the daughters were of the same order.

2.4. Hemodynamic modeling

Resistance was calculated based on vessel geometry through use of standard formulas for resistances in parallel and in series as previously described [15]. Hemodynamic calculations assumed **1**) Poiseuille's law for flow of fluid through a pipe-like structure, **2**) equal pressure at each terminal vessel, and **3**) a correction factor modeling blood viscosity changes in small vessels derived using adult blood [17].

2.5. Statistical analysis

All statistical tests were performed using the R statistical software (www.r-project.org). Data from each group are reported as the mean ± the standard error of the mean (SEM) and analyzed using *t*-tests to compare arteries and veins. A value of $p < 0.05$ was taken to be significant. To assess group differences in the Strahler ordering data, a two-way ANOVA was performed with Strahler order and group (arteries vs. veins) as fixed factors. Where the ANOVA was significant, a Tukey post hoc test was performed for each order.

3. Results

Examination of surface renderings and maximum intensity projection images of arterial and venous specimens (Fig. 1A,D) revealed differences in branching pattern and tree dimensions. A dichotomous branching pattern was evident in arterial specimens (Fig. 1A). The single umbilical artery branched extensively at the surface of the placenta (chorionic plate). These surface vessels further branched into the placenta to become intraplacental arteries that then branch into smaller arterioles and capillaries. The similarly sized single umbilical vein (Fig. 1B) branched into two visually distinctive tree halves near the site of umbilical artery chorionic plate insertion (Fig. 1D). Both halves sat superficial to the arterial vessels, exhibiting a magistral/mixed branching pattern along the chorionic plate [18]. Relative to the arterial vasculature,