Placenta 57 (2017) 137–143

Contents lists available at ScienceDirect

Placenta

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

Vesicular uptake of macromolecules by human placental amniotic epithelial cells

Rita Sharshiner [a], Robert A. Brace [a, b, *], Cecilia Y. Cheung [a, b]

[a] Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, Oregon Health and Science University, Portland, OR, USA
[b] Center for Developmental Health, Oregon Health and Science University, Portland, OR, USA

ARTICLE INFO

Article history:
Received 29 March 2017
Received in revised form
28 May 2017
Accepted 30 June 2017

Keywords:
Amniotic fluid
Amnion
Endocytosis
Fluorescence microscopy
Vesicular transport

ABSTRACT

Introduction: Studies in animal models have shown that unidirectional vesicular transport of amniotic fluid across the amnion plays a primary role in regulating amniotic fluid volume. Our objective was to explore vesicle type, vesicular uptake and intracellular distribution of vesicles in human amnion cells using high- and super-resolution fluorescence microscopy.
Methods: Placental amnion was obtained at cesarean section and amnion cells were prepared and cultured. At 20%–50% confluence, the cells were incubated with fluorophore conjugated macromolecules for 1–30 min at 22 °C or 37 °C. Fluorophore labeled macromolecules were selected as markers of receptor-mediated caveolar and clathrin-coated vesicular uptake as well as non-specific endocytosis. After fluorophore treatment, the cells were fixed, imaged and vesicles counted using Imaris® software.
Results: Vesicular uptake displayed first order saturation kinetics with half saturation times averaging 1.3 min at 37 °C compared to 4.9 min at 22 °C, with non-specific endocytotic uptake being more rapid at both temperatures. There was extensive cell-to-cell variability in uptake rate. Under super-resolution microscopy, the pattern of intracellular spatial distribution was distinct for each macromolecule. Co-localization of fluorescently labeled macromolecules was very low at vesicular dimensions.
Conclusions: In human placental amnion cells, 1) vesicular uptake of macromolecules is rapid, consistent with the concept that vesicular transcytosis across the amnion plays a role in the regulation of amniotic fluid volume; 2) uptake is temperature dependent and variable among individual cells; 3) the unique intracellular distributions suggest distinct functions for each vesicle type; 4) non-receptor mediated vesicular uptake may be a primary vesicular uptake mechanism.

1. Introduction

Of the nearly 4 million births in the US each year, 5%–10% are affected by abnormal amniotic fluid volume (AFV) and, in many cases, the etiology is unknown [1,2]. Both oligohydramnios and polyhydramnios are associated with adverse pregnancy outcomes, including preterm birth, low birth weight, abnormal fetal lie, placental abruption, postpartum hemorrhage and cesarean delivery [2–4]. Presently, there are no effective therapies for treating aberrant AFVs despite efforts to understand AFV regulation.

Experimental studies in sheep suggest that AFV is primarily regulated by intramembranous absorption, that is, the rapid transfer of amniotic water and solutes across the placental amnion into the fetal circulation [5–11]. This intramembranous transport is largely a unidirectional process and is poorly correlated with osmotic gradients, suggesting that vesicular transport across amnion cells is the primary physiologic intramembranous transport mechanism [11–16]. Further, intramembranous solute fluxes increase linearly with volume fluxes, showing that bulk vesicular transport is a major contributor to intramembranous solute absorption [5,12,13,15].

Although electron microscopy studies of human amnion have shown the presence of large numbers of intracellular vesicles and that extracellular fluid uptake is an extremely active process [17],

Abbreviations: AFV, amniotic fluid volume; BSA, bovine serum albumin; CTB, cholera toxin B subunit; Dex, 70 kDa dextran; DIC, differential interference contrast; Tfn, transferrin.

* Corresponding author. Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, L-458 Oregon Health and Science University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97239-3098, USA.
E-mail addresses: sharshin@ohsu.edu (R. Sharshiner), bracer@ohsu.edu (R.A. Brace), cheungce@ohsu.edu (C.Y. Cheung).

http://dx.doi.org/10.1016/j.placenta.2017.06.344
0143-4004/

vesicle type and kinetic characteristics have not been established.

Two types of vesicles that may be involved with receptor-mediated fluid transport are caveolar and clathrin-coated vesicles [18–25]. Caveolae are involved in many biological processes including endocytosis and transcytosis and play a major role in transcellular albumin transport [18,20,26]. Clathrin-coated vesicles endocytose fluid, various proteins, essential nutrients and ions, playing a role in cell homeostasis as well as transcellular transport [27–29]. In addition, there are at least two types of non-receptor mediated vesicles that may be involved in uptake of fluids and macromolecules. These include micropinocytes and macropinocytes [30].

Our objectives were to use specific fluorophore-labeled macromolecules as markers of receptor mediated and non-receptor mediated vesicular uptake [18,20,26,31–36] to 1) determine whether caveolar and/or clathrin-coated vesicles are present in human amnion cells, 2) analyze the kinetic characteristics of vesicle uptake and 3) determine the spatial distribution of vesicles within amnion cells. Placental amnion cells were used for the study as transport of amniotic fluid and solutes into fetal blood occurs across the placental rather than across the reflected amnion [5,16].

2. Materials and methods

2.1. Study population

Participants in this study were pregnant women with a term singleton gestation (≥37 weeks) and normal AFVs undergoing elective cesarean delivery prior to the onset of labor at Oregon Health and Science University in Portland, Oregon. Normal AFV was defined as an amniotic fluid index of 5–25 cm and deepest vertical pocket of 2–8 cm by ultrasound assessment within 7 days of delivery date. Ultrasound assessments were performed by either trained nurses or physicians. Exclusion criteria included multiple gestation, non-English and non-Spanish speaking, age <18 years, known fetal anomalies, labor (defined by documented cervical change and regular contractions on day of delivery), suspected chorioamnionitis, and ruptured fetal membranes. Participants were recruited and informed consents signed at the time of admission for their scheduled cesarean deliveries. Chart abstraction was performed by trained study personnel after tissue collection to obtain maternal and infant characteristics. All data were de-identified after collection. Research protocols and study procedures were approved by the Institutional Review Board at Oregon Health and Science University.

Mean maternal age (n = 7) was 32.6 ± 5.5 (SD) years. Five subjects were Caucasian and 2 were Hispanic. Mean gestational age at time of amnion collection was 39 $^{4/7}$ ± 0 $^{5/7}$ weeks. Median AFI was 9.5 (range 6.5–11.8) cm. Forty three percent of infants were female and the median infant weight was 3459 (range 2920–3890) g.

2.2. Amnion tissue collection

Placental amnion was obtained from 7 subjects immediately upon delivery of the placenta at the time of cesarean section. The placental amnion was isolated by separating it from the underlying chorionic plate and trimming circumferentially 1 cm from the edge of the placenta and medially at least 1 cm from the umbilical cord insertion.

2.3. Amnion cell preparation and culture

Amnion tissue was either used immediately for explant studies or for preparation of amnion cell cultures.

Amnion explants were used initially to determine whether cells in the amnion would take up fluorophore labeled macromolecules and subsequently to examine intercellular vesicular uptake variability by low-resolution imaging. Cultured amnion cells were used to study vesicular uptake kinetics, co-localization and intracellular distribution by high- and super-resolution imaging.

To prepare amnion cell cultures, the membranes were minced, digested with 0.31% trypsin and the dispersed cells were plated onto 4-chamber IbiTreat coverslips (Ibidi USA, Inc., Madison, WI) for high-resolution imaging and Ibidi glass 4-chambered coverslips for super-resolution imaging. Cells were maintained in DMEM/F12 culture medium supplemented with 10% FBS (fetal bovine serum, Thermo Fisher Scientific, Waltham, MA) and 1× antibiotic/antimycotic at 37 °C in a humidified atmosphere of air supplemented with 5% CO_2. At 20%–50% confluence, the cells were used for labeling and imaging studies.

2.4. Amnion explant staining for low-resolution imaging

Fresh amnion was dissected into 10 × 10 mm fragments and incubated at 37 °C in DPBS (Dulbecco's phosphate-buffered saline supplemented with calcium and magnesium, Hyclone Laboratories, Inc., Logan, Utah). At 1 h, the DPBS was replaced with 25 μg/ml BSA-555 (bovine serum albumin Alexa Fluor 555, Thermo Fisher Scientific) in DPBS for 60 min. The BSA-555 was reconstituted, diluted and mixed prior to treatment. Following treatment, the membranes were washed, fixed in cold freshly prepared 4% PFA (paraformaldehyde, Sigma-Aldrich) in DPBS and nuclear counter-stained with NucBlue fixed cell ready probe (DAPI, 4′,6-diamidino-2-phenylindole, Thermo Fisher Scientific). The explants were mounted using Fluoromount G (Southern Biotech, Birmingham, AL) on Superfrost™ microscopy slides (Thermo Fisher Scientific) with #1.5 glass coverslips.

2.5. Amnion cell staining for high-and super-resolution imaging

Cultured cells were serum withdrawn for 1 h and treated with the following fluorophore labeled macromolecules (Molecular Probes, Thermo Fisher Scientific) in DPBS: CTB-488 (15 μg/ml, cholera toxin subunit B, Alexa Fluor 488 conjugate), BSA-488 (25 μg/ml, bovine serum albumin, Alexa Fluor 488 conjugate), BSA-555 (25 μg/ml, bovine serum albumin, Alexa Fluor 555 conjugate), Tfn-555 (20 μg/ml, transferrin, Alexa Fluor 555 conjugate), Dex-594 (15 μg/ml, Dextran 70 kDa MW lysine fixable, Texas Red conjugate). Fluorophore labeled conjugates were diluted to desired concentrations, centrifuged to remove potential particulates, and vortex mixed prior to treatment. These labeled macromolecules were selected as specific makers: CTB for caveolar vesicles, Tfn for clathrin-coated vesicles, BSA for receptor-mediated vesicular uptake and Dex as a marker for non-specific endocytosis including micro- and macropinocytosis [18,20,26,31–36].

For kinetic studies, cultured cells were single labeled with each fluorescent molecule for 1, 3, 10 or 30 min(s). These incubation times were chosen because our preliminary studies showed that uptake saturation occurred in less than 30 min, as illustrated in the results below. After each treatment period, cells were fixed immediately in cold 4% PFA for 45 min, washed and cell nuclei stained with DAPI.

For co-localization experiments, the cells were dual-labeled with the fluorescent molecules for 30 min, fixed, washed, and the cell nuclei stained with DAPI.

2.6. Image acquisition

Amnion explants were viewed on a laser scanning confocal