Differential Effect of Maternal Hypoxia on Syncytiotrophoblast-And Endothelial-Derived Exosomes in an ex Vivo Human Dual-Perfusion System.

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Abstract #0275

Background: Placental oxygen environment is an important regulator of maternal and fetal vascular and metabolic responses. Mechanisms of this response include, beside endocrine and cytokine factors, placental-derived extracellular vesicles (EVs). The aim of this study was to evaluate the effect of hypoxia on the concentration of the different populations of EVs in maternal and fetal compartments using a human dual perfusion system. Methods: We used a human ex vivo dual perfusion technique, which had been modified to normoxic (N, n=3) and hypoxic (H, n=3) conditions, with soluble oxygen tension in maternal inflow (in mmHg): 286 ±7 (N) and 80 ±16 (H), fetal outflow 78.5 ±4.9 and 28.5 ±17 and fetal inflow (N and H respectively). The perfusate was collected at 120 min. The total numbers of particles were quantified in the perfused buffer by nanoparticle tracking analysis (NTA). The different population of vesicles was determined based in their size and classified as <50, 50-150, 150-200 and >200nm. Exosomes were isolated by differential and buoyant density centrifugation and quantified using nanocryostats (Qdot) coupled with CD63 using NTA in fluorescence mode. Results: The total concentration of EVs was significantly higher -8-fold in the maternal compared with fetal compartments. Hypoxia induced the release of EVs in the maternal compartment without showing variation in the fetal compartments. The analyses of the subpopulations of EVs show that hypoxia increased the vesicles between 50-150 nm, 150-200nm and >200nm in 2.2-fold, 1.4-fold and 1.3-fold, respectively. The majority of EVs are >200nm (>80% of the total), however, hypoxia specifically increased the proportion of vesicles between 50-150 nm. Finally, the levels of exosomes (qdot-CD63+) was significantly higher under hypoxia compared to normoxia in the maternal compartment. Conclusions: Placental hypoxia specifically induced the secretion of ST derived maternal, but not endotherial derived fetal exosomes.

Background:

Placental oxygen environment is an important regulator of maternal and fetal vascular and metabolic responses. Mechanisms of this response include, beside endocrine and cytokine factors, placental-derived extracellular vesicles (EVs). We previously described effect of maternal hypoxia in the in vitro placental perfusion model (Fig.1) on the fetal and maternal inflammatory and oxidative stress responses and effect of hypoxia on the extracellular vesicles shedding by the extracellular trophoblast (EVTr) (Fig.2). The aim of this study was to evaluate the effect of maternal hypoxia on the concentration of the different populations of EVs in maternal and fetal compartments using a human dual perfusion system.

Results and Discussion

The total concentration of EVs was significantly higher -8-fold in the maternal compartment compared with fetal compartments. Hypoxia induced the release of EVs in the maternal compartment without showing variation in the fetal compartments. The analyses of the subpopulations of EVs show that hypoxia increased the vesicles between 50-150 nm, 150-200nm and >200nm in 2.2-fold, 1.4-fold and 1.3-fold, respectively. The majority of EVs are >200nm (>80% of the total), however, hypoxia specifically increased the proportion of vesicles between 50-150 nm. Finally, the levels of exosomes (qdot-CD63+) was significantly higher under hypoxia compared to normoxia in the maternal compartment. We previously reported that hypoxic conditions, cause release of specific populations of exosomes by extra villous trophoblast (EVt). Exosomes from EVt in hypoxia (1%) oxygen had micro-RNAs, associated with regulation of inflammatory responses. Interestingly, the inflammatory cytokines were detected in maternal perfusates at 180-360 min after initiation of hypoxic treatment in an ex vivo perfused placenta. The 8-fold difference between fetal and maternal exosomes' content in ex vivo model correlated perfectly with the reported by us data in pregnant non-human primates (IFPA, 2017, Abstract #0281). Absence of changes in fetal exosomal content is surprising, since fetal oxygen content was half of the maternal and fetal values at the beginning of the experiment. The absence of exosomal release under hypoxic conditions from endothelial cells has been described in tumor cells (Proc Natl Acad Sci U S A. 2011 Aug 9;108(32):31347-52), while in HUVEC (human umbilical cord endothelial cells) hypoxic treatment Stimulated vesicular release of ATP (Placenta. 2015 Jul;36(7):759-66). Placental responses to maternal hypoxia might have two stage responses: firstly, immediate response, involving maternal cardiovascular system and secondary, fetal responses.

Materials and Methods

We used a human ex vivo dual placental perfusion technique, which had been modified to maternal normoxic (N, n=3) and hypoxic (H, n=3) conditions, with soluble oxygen tension in maternal inflow (in mmHg): 286 ±7 (N) and 80 ±16 (H), fetal outflow 78.5 ±4.9 and 28.5 ±17 and fetal inflow (N and H respectively). The perfusate was collected after 120 min of perfusion. Release and behavior evaluation of 120 min time period was based on our work (Gandhi et al., IFPA 2016), demonstrating 120 min time frame as the time of detectable changes in perfusate detectable by Raman spectroscopy analyses. The total numbers of particles were quantified in the perfused buffer by nanoparticle tracking analysis (NTA). The different population of vesicles was determined based in their size and classified as <50, 50-150, 150-200 and >200nm. Exosomes were isolated by differential and buoyant density centrifugation and quantified using nanocryostats (Qdot) coupled with CD63 using NTA in fluorescence mode.

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