OBSTETRICS

Evidence that fetal death is associated with placental aging

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BACKGROUND: The risk of unexplained fetal death or stillbirth increases late in pregnancy, suggesting that placental aging is an etiological factor. Aging is associated with oxidative damage to DNA, RNA, and lipids. We hypothesized that placentas at >41 completed weeks of gestation (late term) would show changes consistent with aging that would also be present in placentas associated with stillbirths.

OBJECTIVE: We sought to determine whether placentas from late-term pregnancies and unexplained stillbirth show oxidative damage and other biochemical signs of aging. We also aimed to develop an in vitro term placental explant culture model to test the aging pathways.

STUDY DESIGN: We collected placentas from women at 37-39 weeks' gestation (early term and term), late term, and with unexplained stillbirth. We used immunohistochemistry to compare the 3 groups for: DNA/RNA oxidation (8-hydroxy-deoxyguanosine), lysosomal distribution (lysosome-associated membrane protein 2), lipid oxidation (4-hydroxynonenal), and autophagosome size (microtubule-associated proteins 1A/1B light chain 3B, LC3B). The expression of aldehyde oxidase 1 was measured by real-time polymerase chain reaction. Using a placental explant culture model, we tested the hypothesis that aldehyde oxidase 1 mediates oxidative damage to lipids in the placenta.

RESULTS: Placentas from late-term pregnancies show increased aldehyde oxidase 1 expression, oxidation of DNA/RNA and lipid, perinuclear location of lysosomes, and larger autophagosomes compared to placentas from women delivered at 37-39 weeks. Stillbirth-associated placentas showed similar changes in oxidation of DNA/RNA and lipid, lysosomal location, and autophagosome size to placentas from late term. Placental explants from term deliveries cultured in serum-free medium also showed evidence of oxidation of lipid, perinuclear lysosomes, and larger autophagosomes, changes that were blocked by the G-protein-coupled estrogen receptor 1 agonist G1, while the oxidation of lipid was blocked by the aldehyde oxidase 1 inhibitor raloxifene.

CONCLUSION: Our data are consistent with a role for aldehyde oxidase 1 and G-protein-coupled estrogen receptor 1 in mediating aging of the placenta that may contribute to stillbirth. The placenta is a tractable model of aging in human tissue.

Key words: aging, aldehyde oxidase 1, autophagosome, DNA/RNA oxidation, fetal death, G-protein-coupled estrogen receptor 1, lipid oxidation, placenta, placental explant culture, raloxifene, stillbirth

Introduction

Unexplained fetal death is a common complication of pregnancy occurring in approximately 1 in 200 pregnancies in developed countries[1] and more frequently in the developing world. While no cause has been established, the rate of fetal death rises rapidly as gestation progresses >38 weeks.[2] Johnson et al[3] proposed the operational definition of aging as an increase in risk of mortality with time, which is consistent with a role for aging in the etiology of stillbirth.[4] Supporting this view, a histopathological study of placentas associated with cases of unexplained intrauterine death at term revealed that 91% showed thickening of the maternal spiral artery walls, 54% contained placental infarcts, 10% had calcified areas, and 13% demonstrated vascular occlusion[5]; another study reported increased atherosclerosis,[6] changes associated with aging in other organs. Supporting a link between placental aging and stillbirth, Ferrari et al[7] recently reported that telomere length is reduced in placentas associated with stillbirth. Fetal growth restriction is also associated with both stillbirth and telomere shortening.[8] We therefore sought to determine whether placentas from women who delivered >41 completed weeks (late term) or had stillbirth had biochemical evidence of aging. As markers of aging we chose to measure 8-hydroxy-deoxyguanosine (8OHdG) (a marker of DNA oxidation) and 4-hydroxynonenal (4HNE) (a marker of lipid oxidation) as both have been described to increase in the brain with aging, and the enzyme aldehyde oxidase (AOX), which is known to generate oxidative damage in the kidney. Aging is also known to affect the effectiveness of the intracellular recycling process that involves fusion of acidic hydrolase containing lysosomes with autophagosomes; we therefore sought changes in these intracellular organelles in the late-term placentas and those associated with stillbirth.

EDITORS' CHOICE

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Materials and Methods

Ethics, collection, and processing of tissues

This study was approved by the human research ethics committee of the Hunter New England Health Services and the University of Newcastle, Australia. Human placentas were collected after written informed consent was obtained from the patients by midwives. Placentas were collected from women at 37-39 weeks' gestation undergoing cesarean delivery for previous cesarean delivery or normal vaginal delivery, women at ≥41 weeks' gestation undergoing cesarean

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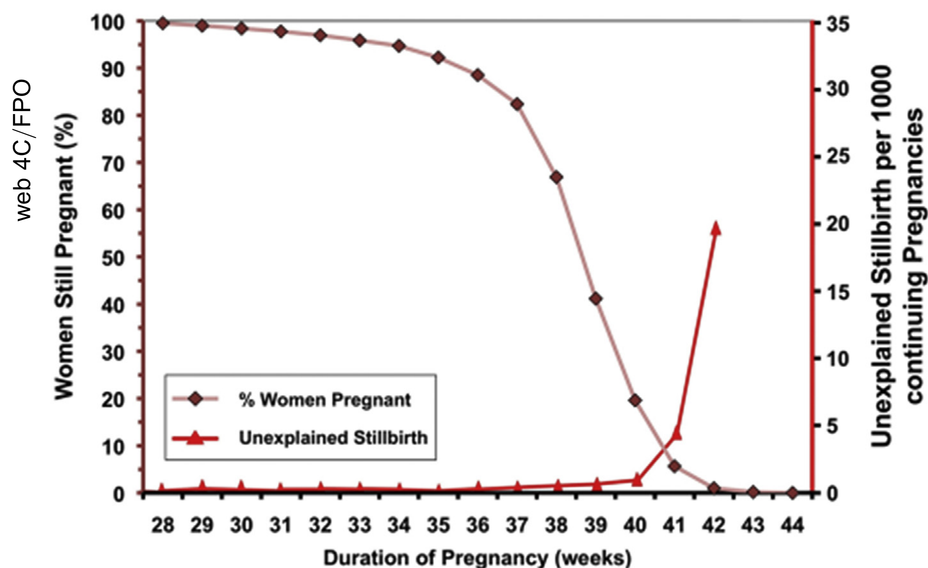
FIGURE 1
Relationship between stillbirth and number of continuing pregnancies



Kaplan-Meier plot of number of continuing pregnancies as function of gestational age and plot of unexplained stillbirth per 1000 continuing pregnancies; data from Omigbodun and Adewuyi[10] and Sutan et al.[2] Plot shows increase in risk of stillbirth with time consistent with operational definition of aging proposed by Johnson et al[3] and relatively small number of pregnancies at risk of stillbirth by 41 weeks because of prior delivery. Reproduced with permission.[4]

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delivery or normal vaginal delivery, and women who had stillborn infants undergoing vaginal delivery. Placentas were collected immediately after delivery and processed without further delay. Villous tissues were sampled from multiple sites and prepared for histology and RNA extraction. For each placenta, tissues were obtained from at least 5 different regions of the placenta and 4-5 mm beneath the chorionic plate. Samples from each individual placenta were immediately frozen under liquid nitrogen and stored at −80°C until subsequent experiments. For histology experiments, tissues were fixed in 2% formaldehyde for 24 hours, stored in 50% ethanol at room temperature, and embedded in paraffin. To create a placental roll a 2-cm strip of chorioamniotic membrane was cut from the periphery of the placenta keeping a small amount of placenta attached to the membrane. The strip was rolled around forceps leaving residual placenta at the center of the cylindrical roll. The cylindrical roll was then cut perpendicular to the cylindrical axis to obtain 4-mm thick sections and fixed in formalin. Placentas from patients with infection, diabetes, preeclampsia, placenta previa, intrauterine growth restriction, or abruption were excluded.

Reagents and antibodies

Antibodies against lysosome-associated membrane protein 2 (LAMP2) and AOX1 were obtained from BD Biosciences (Sydney, Australia) and Proteintech (Rosemont, IL), respectively. Antibody against LC3B and G-protein-coupled estrogen receptor 1 (GPER1) were obtained from Novus Biologicals (Littleton, CO). Antibodies against 8OHdG and 4HNE were purchased from Abcam (Melbourne, Australia). Dulbecco modified Eagle medium (DMEM), antibiotic-antimycotic, Nupage precast 12-well protein gel, and prolong gold antifade mounting media with 4′,6-diamidino-2-phenylindole (DAPI), Alexa conjugated secondary antibodies were obtained from Thermo Fisher Scientific Australia Pty (Melbourne, Australia). The horseradish peroxide conjugated secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA). Fetal bovine serum (FBS) was obtained from Bovogen Biologicals Pty Ltd (Melbourne, Australia). Protease inhibitor and phosphatase inhibitor were supplied by Roche (Sydney, Australia). Raloxifene was purchased from Sigma-Aldrich (Sydney, Australia) and G1 was supplied by Tocris-Bioscience (Bristol, United Kingdom). The BCA protein assay kit was obtained from Thermo Fisher Scientific Australia Pty. All other chemicals were purchased from either Ajax Finechem Pty Ltd (Sydney, Australia) or Sigma-Aldrich.

Placental explant culture

For in vitro experiments, human term placentas (all at 39 weeks of gestation) were obtained from women with normal singleton pregnancies without any symptoms of labor after an elective (a scheduled repeat) cesarean delivery. Placentas were collected immediately after delivery and prepared for explant culture. Villous tissues of placentas were randomly sampled from different regions of the placenta 4-5 mm beneath the chorionic plate. Tissues were washed several times with Dulbecco phosphate-buffered saline under sterile conditions to remove excess blood. Villous explants of ∼2 mm^3 were dissected and placed into 100-mm culture dishes (30 pieces/dish) containing 25 mL of DMEM supplemented with 2 mmol/L L-glutamine, 1% Na-pyruvate, and 1% penicillin/streptomycin (100×) solution with the addition of 10% (vol/vol) FBS and cultured in a cell culture chamber at 37°C temperature under 95% air (20% oxygen) and 5% carbon dioxide

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