OBSTETRICS

Cardiac dysfunction is associated with altered sarcomere ultrastructure in intrauterine growth restriction

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OBJECTIVE: The purpose of this study was to assess whether abnormal cardiac function in human fetuses with intrauterine growth restriction (IUGR) is associated with ultrastructural differences in the cardiomyocyte sarcomere.

STUDY DESIGN: Nine severe early-onset IUGR fetuses and 9 normally grown fetuses (appropriate growth for gestational age) who died in the perinatal period were included prospectively. Cardiac function was assessed by echocardiography and levels of B-type natriuretic peptide and troponin-I. Heart sections were imaged by second harmonic generation microscopy, which allowed unstained visualization of cardiomyocyte's sarcomere length.

RESULTS: Echocardiographic and biochemical markers showed signs of severe cardiac dysfunction in IUGR fetuses. Second harmonic generation microscopy demonstrated a significantly shorter sarcomere length in IUGR as compared with appropriate growth for gestational age fetuses.

CONCLUSION: IUGR is associated with changes in the cardiomyocyte contractile machinery in the form of shorter sarcomere length, which could help to explain the cardiac dysfunction previously documented in IUGR.

Key words: cardiac function, cardiomyocyte, intrauterine growth restriction, sarcomere, second harmonic generation microscopy

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Intrauterine growth restriction (IUGR) affects 7-10% of all pregnancies and constitutes an important cause of perinatal death and long-term morbidity.[1-3] Epidemiologic evidence has long suggested a link between low birthweight and increased cardiovascular death in adulthood.[4] Recent studies have demonstrated that IUGR fetuses present cardiovascular dysfunction in utero[5-8] that persists postnatally in the form of cardiovascular dysfunction and remodelling.[9,10] However, the molecular mechanisms underlying the relationship between IUGR and increased risk of cardiovascular disease are understood poorly.[11]

The basic unit of cardiomyocyte's contractile machinery is the sarcomere. Disruption of normal sarcomere structure is involved in cardiac dysfunction and remodeling in a substantial number of cardiac conditions such as inherited cardiomyopathies,[12,13] anthracyclines cardiotoxicity,[14] and heart failure.[15] Second harmonic generation microscopy (SHGM) is a recent technique that is based on a nonlinear optical effect known as second harmonic generation. SHGM is used widely in biomedical research as an imaging technique that allows the measurement of morphologic features at subcellular level, including sarcomere length and pattern[16,17] by identification of the myosin of thick filaments of sarcomere.[18,19] Recently, our group has validated an automated method to better quantify sarcomere length in the fetal heart.[20]

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We hypothesized that cardiac dysfunction in IUGR could be associated with disruption of normal sarcomere structure. Within a large prospective research program to characterize cardiac function in IUGR,[9] we conducted a nested case-control study. Cardiac tissue was retrieved from 9 IUGR fetuses who died around delivery and from 9 appropriate growth for gestational age (AGA) fetuses who were selected from patients who underwent termination of pregnancy. Cardiac tissue was analyzed by means of SHGM, and sarcomere length was assessed in cases and control subjects.

Materials and Methods

Study groups

The study population included 9 severe early-onset IUGR and 9 AGA fetuses from 19-33 weeks of gestation who died in the perinatal period because of perinatal complications or medical termination of pregnancy. Cases were singleton pregnancies with IUGR that had been included in a large prospective cohort that evaluated cardiac dysfunction in IUGR.[9] AGA fetuses were selected among women who underwent termination of pregnancy because of severe maternal disease or noncardiac malformations. Intracardiac injection of potassium chloride was performed electively to induce cardiac arrest before induction of labor according to local standard protocols in those cases that undergo termination of pregnancy after 22 weeks.

The study protocol was approved by the Ethics Committee at the participating institution, and all patients provided written informed consent. Exclusion criteria were chromosomal anomalies or evidence of fetal infection. In all pregnancies, gestational age was calculated based on the crown-rump length at the first-trimester ultrasound examination. *IUGR* was defined as an estimated fetal weight at <10th percentile according to local reference curves[21] together with umbilical artery (UA) pulsatility index (PI) at >2 standard deviations.[22]

Fetal ultrasound assessment

All cases underwent ultrasonographic examination of fetal well-being and hemodynamics within 48 hours of delivery or fetal death, including complete morphologic examination, fetal weight, UA, middle cerebral artery, and cerebroplacental ratio. Ultrasound assessment was performed with a Siemens Sonoline Antares (Siemens Medical Systems, Erlangen, Germany) or a Voluson 730 Expert (GE Medical Systems, Milwaukee, WI) with 6-4 or 6-2 MHz curved array probes. All Doppler estimations were done in the absence of fetal body movements and, if required, with maternal voluntary suspended respiration. The angle of insonation was kept at <30°, and the wall filter was set to 70 Hz to avoid sound artifacts. The mechanical and thermal indices were maintained at <1. UA PI was obtained from a free loop of the umbilical cord. Middle cerebral artery PI was measured in a transverse view of the fetal skull at the level of its origin from the Circle of Willis. Cerebroplacental ratio was calculated as middle cerebral artery P/UA PI.[21] All individual Doppler data were normalized by conversion of the measurements into z-scores (standard deviation from the gestational age mean).[22]

Fetal echocardiography

Echocardiographic measurements performed within 48 hours of delivery or fetal death included ductus venosus PI, left myocardial performance index (MPI), and diastolic ventricular filling ratios. Ductus venosus PI was obtained from a mid-sagittal or alternatively transverse section of the fetal abdomen.[23] Left MPI was obtained in a cross-sectional image of the fetal thorax and an apical 4-chamber view, placing the Doppler sample volume on the medial wall of the ascending aorta including the aortic and mitral valve. The movements (clicks) of the valves in the Doppler trim were used as landmarks to calculate the isovolumetric contraction and relaxation time, and the ejection time. MPI was calculated as (isovolumetric contraction + relaxation time)/ejection time.[24-27] Mitral and tricuspid diastolic ventricular filling ratios were obtained in an apical 4-chamber view, with the Doppler sample volume just below the atrioventricular valves, and were calculated by division of early diastolic by atrial peak inflow velocities.[25] Echocardiographic parameters were normalized into z-scores.[23-25]

Cardiovascular markers in fetal blood

Fetal umbilical ethylenediaminetetraacetic acid–treated blood was obtained from the umbilical vein after cord clamp at delivery in cases or at cardiocentesis in fetuses who underwent termination of pregnancy. All samples were processed within 1 hour. Plasma was separated by centrifugation at 1400*g* for 10 minutes at 4°C; samples were stored immediately at −80°C until assay. Cord blood levels of B-type natriuretic peptide (BNP) were measured with an immunoassay system (ADVIA Centaur BNP; Siemens Healthcare Diagnostics, Deerfield, IL), as described previously.[28] Troponin-I levels were measured with commercially available assays from Centaur CP–troponin-I assay (Siemens Healthcare Diagnostics).

Heart tissue collection

Heart samples were collected at autopsy. A transverse section of the upper third of posterior left ventricular wall was obtained and then fixed in 3.7% paraformaldehyde, dehydrated, and embedded in paraffin. Subsequently, 30-μm–thick sections were cut from the paraffin blocks for microscopic examination with a Leica microtome (Leica RM 2135; Leica Microsystems Heidelberg GmbH, Manheim, Germany). Finally, sections were mounted onto Silane- (Sigma-Aldrich, St. Louis, MO) coated thin slides. Tissue sections were deparaffined with xylene, hydrated with decreasing concentrations of ethanol (100°/96°/70°), and finally covered with Mowiol 4-88 mounting medium (Sigma-Aldrich). All samples were processed and imaged by the same person (A.G.-T.).

SHGM of heart tissue

Detection of SHGM was performed with a Leica TCS-SP5 laser scanning spectral confocal multiphoton microscope (Leica Microsystems Heidelberg GmbH) equipped with a Near Infrared laser (Mai Tai Broad Band 710-990 nm, 120 femptosecond pulse) and DMI6000