Death, dying, and exhaustion in the ductus arteriosus: prerequisites for permanent closure

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CENTRAL SHUNTS EXIST IN THE fetal vascular system to enable circulation of enriched fetal blood. Delivery of metabolic wastes and gases to the placenta and return of oxygen and essential nutrients after exchange with maternal blood supply rely on these unique vascular channels. The ductus arteriosus is a large fetal vascular shunt situated between the pulmonary artery and aorta. It serves as the primary conduit for freshly oxygenated blood, which bypasses the underinflated fetal lung and supplies oxygen and nourishment to fetal end organs. Preservation of ductal patency in utero is essential for fetal viability. On the other hand, constriction of the ductus arteriosus must occur soon after birth to reroute right ventricular output into the newly inflated lung, providing a pulmonary rather than placental site for gas exchange in the newborn. Complete occlusion of the ductus arteriosus lumen and closure of the foramen ovale establishes the mature, divided circulatory pattern with separate pulmonary and systemic vascular systems.

Failure of timely ductus arteriosus closure leaves the newborn vulnerable to pulmonary overcirculation and diminished systemic blood flow. If unrecognized or left untreated, a patent ductus arteriosus (PDA) in premature neonates can contribute to intraventricular hemorrhage, pulmonary edema, respiratory failure, renal insufficiency, and necrotizing enterocolitis (2, 4). Prolonged duration of the PDA places the infant at even greater risk, including increased incidence and severity of bronchopulmonary dysplasia and the development of excessive pulmonary vascular remodeling, pulmonary hypertension, and eventual heart failure.

It is remarkable that this vessel behaves in such a different manner than the great arteries it connects. The ductus is distinguished from the surrounding vasculature by its embryologic derivation from the left sixth aortic arch, the contribution of migratory neural crest cells, and exquisite sensitivity to oxygen tension (1, 17). Development of a muscular phenotype compared to surrounding elastic arteries provides the structural and biomechanical basis for its rapid constriction after birth. Yet the molecular pathways that underlie ductus constriction and permanent closure after birth are not completely understood.

In this issue, Levin, Clyman, and colleagues (12) address new potential etiologies for failure of ductus arteriosus closure in prematurely born infants. Ductal closure is considered to occur in two interrelated phases: reversible, “functional” constriction of the vessel that begins soon after birth, and irreversible “anatomic” closure that results in permanent remodeling of the vessel into a fibrous ligament that persists through adult life. Ineffective ductal closure in preterm infants is partially explained by decreased sensitivity to the contractile effects of oxygen, diminished responsiveness to prostaglandin withdrawal at birth, increased levels of prostaglandins, nitric oxide, and other vasodilatory stimuli, and immaturity of the structural apparatus necessary for constriction and obliteration of the ductus lumen (6, 15). Prior studies from the Clyman laboratory (3, 5, 8, 9) have elegantly demonstrated the presence of hypoxia and ischemia in the thickened muscular wall of the closing ductus of animals born at term gestation. Hypoperfusion of the vasa vasorum combined with increasing diffusion distance resulted in circumferential watershed-like areas of cellular compromise in the midst of the medial wall. Apoptosis of medial smooth muscle cells occurred during permanent closure and remodeling of the term ductus but was reduced in the ductus of immature animals. A link between ATP depletion and apoptosis was recently postulated (11). These studies set the stage for the current investigation by Levin et al. (12) on the role of apoptosis and cellular metabolism in the ductus arteriosus of premature baboons.

Term and preterm baboons with successful ductus closure by 6 days of age had similar increases in markers of tissue hypoxia. Not surprisingly, preterm animals with incomplete ductus constriction showed an intermediate degree of vessel hypoxia. Hypoxia markers in the patent premature ductus were statistically different than fetal vessels, however, indicating that the preterm ductus with incomplete closure still experiences a significant degree of hypoxia. The more interesting result came from the correlation of these findings with apoptosis and changes in glucose, glycogen, and ATP. Using an in situ bioluminescence enzyme assay, the authors showed that the premature baboon ductus undergoes similar reductions in ATP and glucose as the term ductus, if the preterm vessel is able to accomplish complete closure. Changes in glycogen concentration were less consistent; reduced levels were noted in the patent preterm ductus, but little or no change was noted in the successfully closed ductus of either term or preterm animals. As shown by dUTP nick-end labeling (TUNEL) assay, the closed preterm ductus had a marked increase in the number of positively staining nuclei, while the persistently patent preterm ductus revealed minimal staining. A strong correlation was noted between TUNEL staining and ATP and glucose depletion in both term and preterm vessels. A relationship between apoptosis and glycogen was only noted in the term ductus. Significant correlation was noted between the size of the ductus lumen and each experimental parameter, with the notable exception of glycogen depletion in the preterm ductus.

From these studies, it appears that successful closure of the preterm ductus arteriosus depends on apoptosis and depletion of certain cellular energy stores, similar to the term ductus. The persistently patent preterm ductus had less hypoxia and apoptosis than vessels that managed to close. Despite the beauty of these studies, a chicken-and-egg argument remains, because it is unclear whether the ductus fails to close due to these events or whether the cellular changes are not as striking because the...
vessel has not fully constricted in the first place due to other mechanical or molecular reasons, as yet unexplained. Fortunately, the authors addressed this by performing another set of experiments showing that ductal rings incubated in glucose-deficient Krebs buffer were less contractile and developed lower ATP concentrations than rings incubated in glucose-replete solution. These experiments begin to show the important causal relationship of energy depletion and cell death to effective ductal constriction and remodeling.

Apoptosis in the closing ductus arteriosus has been demonstrated by light and electron microscopy, DNA laddering, TUNEL staining, and other techniques (5, 7, 10, 14, 16). Clyman and others have previously shown that cell death in the ductus does not depend on caspase-3, caspase-7, or Bcl pathways (5, 10), suggesting that cell turnover during ductal closure may occur by unique pathways. The microtubule-associated protein LC-3 is involved in fibronectin-dependent smooth muscle function in the ductus (18) but also mediates autophagy or type II programmed cell death, a process that may also play a role in closure of the ductus arteriosus (13). Thus, elucidation of the underlying mechanisms of cell death in the closing ductus arteriosus may shed new light into the causes of persistent ductal patency in newborn infants. The current report by Levin et al. (12) combined with their earlier results provides both in vivo and in vitro data on the critical requirement for nutrient depletion in apoptosis and induction of permanent closure of the term and preterm ductus arteriosus.

REFERENCES