Maternal Vitamin A Administration and the Fetal Ductus Arteriosus

Commentary on the article by Wu *et al.* on page 747

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Patent ductus arteriosus (PDA) continues to be a challenging problem in premature infants, with a prevalence of approximately 30% in infants weighing 500–1500 g (1). The presence of a PDA can complicate the pulmonary course of the low-birth-weight infant, prolonging the need for mechanical ventilatory support and contributing to the pathogenesis of chronic lung disease or bronchopulmonary dysplasia. PDA is also associated with increased incidence of intraventricular hemorrhage, necrotizing enterocolitis, and nutritional deprivation (2). Current management strategies employing either indomethacin therapy or surgical ligation are not without potential complications, and reopening of the ductus is a frequent occurrence with nonsurgical approaches (3). As a result, there continues to be a dire need for new mechanistic information about the processes that contribute to failure of ductal closure in the preterm infant.

In the full-term infant, ductal closure occurs in two mechanistic phases. During the first few hours after birth there is functional closure of the ductus lumen due to constriction of the ductal smooth muscle. Functional closure is then followed over the next several days by anatomic closure involving dramatic neointimal hyperplasia and smooth muscle cell loss from the internal aspects of the medial layer. Recent work by Clyman and colleagues indicates that the initial loss of luminal blood flow with constriction produces a zone of hypoxia within the medial layer that may be required for ultimate irreversible anatomic closure (4). Numerous studies suggest that the early ductal constriction, which occurs primarily in response to increased oxygenation, is necessary for anatomic occlusion to follow, and that the constriction with oxygenation is attenuated in the preterm ductus (2, 5–9).

In the present issue of *Pediatric Research,* Wu and colleagues report the findings of studies of the effects of maternal vitamin A administration on the maturation of ductal contraction to oxygen in the fetal rat (10). In experiments performed with isolated ductus arteriosus segments, they found that maternal vitamin A treatment 1–2 days before ductus harvest at 19–21 d of gestation (term = 22 d) accelerated the development of oxygen-induced ductal contraction. The effect of vitamin A was particularly robust when assessed at 19 d of gestation after 2 days of administration. Although the underlying mechanisms may be complex and are yet to be clarified, the biologic and clinical implications of their observations are quite exciting.

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Under normal conditions, closure of the mature ductus to oxygen involves smooth muscle contraction due to increases in intracellular calcium concentrations (11–12). Changes in the activity of potassium channels, either ATP-regulated or direct rectifying potassium channels, and resultant effects on membrane polarity may play a role in the oxygen-induced rise in intracellular calcium (12, 13). In an effort to explain the effects of vitamin A in their model, Wu and coworkers present a logical series of studies evaluating many of these mechanisms in ductus segments equilibrated under low oxygen conditions (0% O_2, pO_2 = 22–25 mm Hg). In the control group at 19 d of gestation, there was no contraction observed with exposure of the ductus to increasing levels of oxygenation in the organ chamber, and oxygen responses at 20 and 21 d of gestation were modest until O_2 levels were 30% (pO_2= 218–232 mm Hg) or greater. In contrast, in the vitamin A-treated group, contraction occurred at 19 d of gestation at all levels of increased O_2 tested, and oxygen responses to 5% O_2 (pO_2= 57–60 mm Hg) at 20 and 21 d were significantly enhanced compared with controls. In a parallel manner, oxygen did not affect intracellular calcium in 19-d controls, but there were increases in intracellular calcium in the ductal wall in response to O_2 at 19-d gestation after vitamin A treatment. However, ductal contraction with membrane depolarization caused by high KCl was unaffected by maternal vitamin A administration. The latter observations suggest that the effects of 1–2 days of maternal vitamin A administration were not related to changes in the sensitivity to calcium or in contractile protein expression, but those specific parameters were not assessed by the authors. Furthermore, norepinephrine-induced contraction and norepinephrine-stimulated increases in intracellular calcium evaluated in the absence of extracellular calcium were also unchanged after vitamin A; since these measurements reflect calcium release from intracellular stores, calcium stores were evidently also unaffected by vitamin A. These cumulative findings suggest that the principal effects of 1- to 2-day long maternal vitamin A administration are on oxygen sensing mechanism(s) and not on the contractile machinery of the ductus arteriosus.

Wu and coworkers provide additional information about the cellular localization of oxygen sensing in the fetal rat ductus as well as the site of the vitamin A effects. In ductus from control animals, similar oxygen-induced contraction was noted in rings lacking endothelium and rings with endothelium intact, indicating that the principal site of oxygen sensing is the medial layer. Furthermore, the enhancement in oxygen sensing after

vitamin A in either the 19-d or 20-d ductus was completely unaffected by endothelium removal. These observations suggest that future investigations of the effects of vitamin A on oxygen sensing in this model should be focused primarily on the ductal smooth muscle. However, the potential role of the endothelium should not be entirely ignored because rat aortic segments cultured in vitamin A-supplemented medium display increased tension development but this effect is lost if the segments are denuded of endothelium before culture (14).

Vitamin A exerts its functions through oxidized metabolites of retinol. The first metabolite, retinaldehyde, is a component of the visual pigment rhodopsin, which is isomerized in response to light to initiate a phototransduction cascade leading to signal transmission to neurons of the optic nerve. The second metabolite, retinoic acid, is a lipid-soluble hormone that controls gene transcription through receptor-mediated events. The first human retinoic acid receptor, RARα, was isolated in 1987 and was found to be activated by *all-trans*-retinoic acid. Other subtypes (RARβ and RARγ), which bind the same ligand, were isolated shortly thereafter. A second class of receptors are designated retinoid X receptors (RXR)α, -β, and -γ, and although they may bind *all-trans*-retinoic acid, their ligand was identified as 9-*cis*-retinoic acid (15).

The role of vitamin A metabolites and their receptors in cardiopulmonary development has been elucidated in an elegant manner over the past 10 years in studies of RAR or RXR null mutant mice. When the mutation affects only one receptor, the mice survive and the abnormalities are limited. However, compound null mutants lacking two or more receptor isoforms have displayed myocardial hypoplasia, anomalies of the aortic arches, and lung hypoplasia (15). In certain cases there has been complete lack of the ductus arteriosus (16, 17). These findings mimic those of vitamin A deficiency which were reported 50 years ago (18, 19). In addition, mice carrying a retinoic acid response element-lacZ transgene, which expresses beta-galactosidase in response to endogenous retinoic acid, display a strong beta-galactosidase signal in the ductus arteriosus from late midgestation into neonatal life. Furthermore, the mature isoform of vascular smooth muscle myosin SM2 is coexpressed in the ductus with the retinoic acid-mediated beta-galactosidase signal (20). Cell culture studies indicate that retinoic acid-mediated signals can induce a smooth muscle phenotype in multipotential cells, and that retinoic acid maintains the differentiation status of cultured vascular smooth muscle cells (21–23). Thus, there is considerable prior evidence of a key role for long-term effects of vitamin A metabolites on cardiopulmonary morphogenesis and vascular smooth muscle differentiation. The current studies by Wu *et al.* now indicate that there are also relatively short-term (1–2 d) effects of vitamin A on the fetal ductus that are of considerable functional significance.

Many questions remain about the mechanisms by which short-term maternal vitamin A administration alters oxygen-induced contraction in the fetal ductus arteriosus. It is yet to be determined if vitamin A modifies the effect of O_2 on the production of vasoactive compounds by the ductus. Wu and colleagues report that indomethacin-induced contraction was enhanced after vitamin A, and this could be due to changes in mechanisms mediating prostaglandin production or action in the ductus. Considering the potential importance of endothelin and nitric oxide to ductal function (24, 25), further studies of the impact of vitamin A on the processes responsible for their production and effects are also warranted. Such endeavors could be readily approached using the experimental paradigm that these authors report.

There are also lingering questions related to the clinical implications of the studies by Wu *et al.* Along with the cardiovascular effects noted above, vitamin A is necessary for normal lung growth and the maintenance of the integrity of the respiratory epithelium. Extremely low-birth-weight infants have low vitamin A status at birth and this has been associated with an increased risk of developing chronic lung disease (26). These observations have led to studies of postnatal vitamin A supplementation in extremely low-birth-weight infants. In a large multicenter randomized trial of postnatal vitamin A supplementation for 4 weeks, there was a reduction in the biochemical evidence of vitamin A deficiency and a slight decrease in the risk of chronic lung disease (27). However, the relationship between vitamin A status at birth and the absence or presence of PDA in preterm infants, and also the effect of postnatal vitamin A administration on the incidence and severity of PDA are currently unknown (26). In light of the findings of Wu and colleagues, further studies are indicated in human preterm infants to answer these questions. It is only through a multifaceted approach in both animal models and humans that the biologic effects of vitamin A on ductal function will potentially be harnessed to impact upon the clinical problem of PDA in the preterm infant.

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