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Nitric oxide activity through guanylate cyclase and phosphodiesterase modulation is impaired in fetal lambs with congenital diaphragmatic hernia☆

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Abstract

Background: Congenital diaphragmatic hernia (CDH) is associated with pulmonary hypertension and death. Administration of nitric oxide (NO) alone remains ineffective in CDH cases. We investigated in near full-term lambs with and without CDH the role of guanylate cyclase (GC), the enzyme activated by NO in increasing cyclic 3′-5′-guanylosine monophosphate, and the role of phosphodiesterase (PDE) 5, the enzyme-degrading cyclic 3′-5′-guanylosine monophosphate.

Methods: Congenital diaphragmatic hernia was surgically created in fetal lambs at 85 days of gestation. Pulmonary hemodynamics were assessed by means of pressure and blood flow catheters (135 days). In vitro, we tested drugs on rings of isolated pulmonary vessels.

Results: In vivo, sodium nitroprusside, a direct NO donor, and methyl-2(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridinylmethoxy)-4-(3,4,5trimethoxyphenyl)-3-isoquinoline carboxylate sulfate (T-1032) and Zaprinast, both PDE 5 blockers, reduced pulmonary vascular resistance in CDH and non-CDH animals. The activation of GC by sodium nitroprusside and the inhibition of PDE 5 by T-1032 were less effective in CDH animals. In vitro, the stimulation of GC by 3(5′hydroxymethyl-2′furyl)-1-benzyl indazole (YC-1) (a benzyl indazole derivative) and the inhibition of PDE 5 by T-1032 were less effective in pulmonary vascular rings from CDH animals. The YC-1–induced vasodilation in rings from CDH animals was higher when associated with the PDE 5 inhibitor T-1032.

Conclusions: Guanylate cyclase and PDE 5 play a role in controlling pulmonary vascular tone in fetal lambs with or without CDH. Both enzymes seem to be impaired in fetal lambs with CDH.

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In instances of congenital diaphragmatic hernia (CDH), both lungs may be hypoplastic with associated hyperplasia of the pulmonary vascular smooth muscle cells, which play a main role in maintaining pulmonary vascular resistance (PVR) and therefore blood flow [1,2]. Congenital diaphragmatic hernia is often associated with hypoxemia, acidosis, heart failure, and death. Administration of nitric oxide (NO), a powerful pulmonary vasodilator, alone remains ineffective in CDH cases [3-5].

Endothelial cells are capable of producing NO by activation of the enzyme NO synthase (NOS). Nitric oxide diffuses toward the vascular smooth muscle and activates soluble guanylate cyclase (GC), thereby increasing the level of cyclic 3′-5′-guanylosine monophosphate (cGMP) [6]. The cGMP-dependent proteine kinase causes vasodilation [7]. Cyclic 3′-5′-guanylosine monophosphate is inactivated by the enzyme cGMP-specific phosphodiesterase (PDE) [6,8].

Postnatal pulmonary vascular tone is influenced by vasoconstrictors, such as endothelin-1 or thromboxane A_2, or vasodilators, such as NO, prostacycline, acetylcholine, or ventilation [9-13]. The presence of NOS in fetal lambs has already been demonstrated [14].

To establish that GC and/or PDE 5 play a key role in the modulation of pulmonary vascular tone at birth, we studied the effect of sodium nitroprusside (SNP), an NO donor; 3(5′ hydroxymethyl-2′furyl)-1-benzyl indazole (YC-1), a benzyl indazole derivative involved in GC activation; and methyl-2(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridinylmethoxy)-4-(3,4,5trimethoxyphenyl)-3-isoquinoline carboxylate sulfate (T-1032) and Zaprinast, both inhibitors of PDE 5. We hypothesized that alterations in NO/GC or cGMP/PDE 5 pathways may contribute to persistence of pulmonary hypertension.

1. Methods

The study and surgical procedure was approved by the Animal Care and Use Committee of the Ecole de Chirurgie, Assistance Publique-Hôpitaux de Paris (France). All animals received humane care in compliance with the European Convention on Animal Care. The operations were performed on pregnant ewes (bred in the “Préalpes du Sud”).

1.1. In vivo experiment: surgical procedure

The anesthesia was induced with pentobarbital. The ewes were ventilated with room air (BIRD Corporation, Palm Spring, CA). Heart rate (HR) and blood oxygen saturation were continuously recorded (M1165 A., Model 54S; Hewlett Packard, Northeastern, France).

1.1.1. First surgical procedure: creation of a diaphragmatic hernia

At 85 days (term, 147 days) of gestation, fetal lambs were delivered through a midline incision of the uterus [15]. The left lung was exposed, and a 2-cm incision was made in the left part of the diaphragmatic muscle. The stomach was pulled into the chest manually. After fetal chest closure, the lamb was gently replaced in the uterus, which was then closed.

1.1.2. Second surgical procedure

Surgery was performed at 135 days. The first steps in the surgical procedure were the same as those described above. Polyvinyl catheters were inserted into the axillary artery and the axillary vein and pushed into the aorta (Ao) and the superior vena cava, respectively. Three catheters were inserted by puncture: a Tygon 22 gauge (G) 10 mm into the main pulmonary artery, a Tygon 20 G 20 mm into the left pulmonary artery (LPA), and a Tygon 20 G 13 mm into the left atrium. Two cuff-type ultrasonic flow probes (6.0 mm; Transonics, Ithaca, NY) were placed around the Ao and the LPA. The probes were attached to an internally calibrated flowmeter. Umbilical-placental circulation was preserved. The position of the catheters and the presence of the diaphragmatic hernia were confirmed at autopsy.

1.1.3. Physiological measurements

Blood pressure in the main pulmonary artery, left atrium, and Ao (pulmonary arterial pressure [PAP], left atrium pressure [LAP], aortic blood pressure [PAo], respectively) was measured with a transducer (Baxter, Bentley Laboratories, Uden, The Netherlands). *Pulmonary vascular resistance* was defined as the difference between the PAP and the LAP divided by the LPA blood flow (mean PAP − mean LAP/LPA blood flow). The blood flow in the Ao and in the LPA (blood flow in the aorta [QAo] and blood flow in the left pulmonary artery [QAP], respectively) was measured with an ultrasonic flow transducer connected to a calibrated flowmeter (T206; Transonic Systems Inc, 6222 NW Maastricht, The Netherlands). Fetal blood samples were obtained every 20 minutes to measure the blood gases (Radiometer Copenhagen; Radiometer A/S, Emdrupves 72 DK, 2400 Copenhagen). pH was kept between 7.35 and 7.45, and blood glucose level was considered normal between 3.5 and 7 mmol/L.

1.1.4. Experimental records

Different protocols were performed using these different drugs on the same animal. The sequence of the drugs was not randomized. Physiological hemodynamic measurements were recorded during 20 seconds every 2 minutes throughout the entire 10-minute procedure and then 20 minutes after the procedure. The period of recovery allowed between drugs was at least 1 hour to return to base line.

Protocol 1. Effect of infusion during 10 minutes of SNP (100 μg) in near full-term lambs with (n = 7) and without CDH (n = 4).

Protocol 2. Effect of infusion during 10 minutes at a rate of 0.5 mL/min of T-1032 (300 μg) in near full-term lambs with (n = 7) and without CDH (n = 4).