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Tracheal occlusion and ventilation changes the nitric oxide pathway in congenital diaphragmatic hernia model

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ABSTRACT

Background: Congenital diaphragmatic hernia (CDH) is associated with lung hypoplasia and pulmonary hypertension. Tracheal occlusion (TO) stimulates fetal lung growth and maturation and reverse vascular changes responsible for pulmonary hypertension, which are related to mechanisms involving nitric oxide (NO) in CDH. We aim to evaluate the effect of TO and ventilation on NO pathways.

Methods: Eight groups were created: (1) control; (2) control ventilated (CV); (3) CDH (CDH); (4) CDH ventilated (CDHV); (5) TO control; (6) TO ventilated; (7) TO + CDH; and (8) TO + CDH ventilated (CDHTOV). Fetuses were weighed, and volume ventilated for 30 min after harvested. Total lung weight and the ratio of total lung weight to body weight, thickness of the middle layer of the pulmonary arteriole, and the air space diameter were measured. The NO synthase inducible and NO synthase inducible were performed by immunohistochemistry and Western blotting.

Results: The total lung weight and the ratio of total lung weight to body weight decreased in animals with nitrofen and also after ventilation for all groups ($P < 0.05$). The thickness of the middle layer of the pulmonary arteriole decreased in all groups with TO when compared with controls ($P < 0.001$). The air space diameter decreased after ventilation in the CDHTOV compared to the TO + nitrofen–induced CDH ($P < 0.001$). Compared to nonventilated cohorts, NO synthase inducible increased in CV and TO ventilated ($P < 0.001$) and decreased in CDHV and CDHTOV ($P < 0.001$). NO synthase inducible increased in CV and CDHV ($P < 0.001$) and decreased in the TO control and CDHTOV ($P < 0.001$).

Conclusions: TO and ventilation alter the NO pathway with possible implications in reducing the pulmonary hypertension in CDH.

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Introduction

Congenital diaphragmatic hernia (CDH) is a well-described condition that occurs in 1 in 2500 live births.[1] An estimated 1 in 2000 pregnancies with CDH-afflicted fetuses do not progress to term secondary to prenatal complications, and despite therapeutic advances, postnatal mortality remains high (32%-62%) due to lung hypoplasia and pulmonary hypertension.[2-5]

Current therapeutic options include fetal intervention with fetoscopic endoluminal tracheal occlusion (TO), postnatal repair, and various neonatal management strategies to minimize morbidity from pulmonary hypertension and lung hypoplasia, for example, surfactant, vasodilators, gentle or high frequency ventilation, and extracorporeal membrane oxygenation (ECMO).[6-8] In particular, prenatal treatment with fetoscopic TO prevents egress of the physiologic fluid produced by the lungs. This mechanical expansion is hypothesized, in part, to improve lung volumes, accelerate lung maturity, and decrease pulmonary hypertension. In patients with severe CDH, where the mortality rate is near 100%, TO has resulted in increased survival in approximately 40%-50% of fetuses.[9]

The pathophysiologic remodeling of the pulmonary vasculature observed in CDH is responsible for the pulmonary hypertension seen clinically in the neonate.[10] One of the agents believed to be involved in these developmental changes is nitric oxide (NO), which is an endogenous molecule important in the regulation of vasomotor tone in vessel wall interactions, replication of smooth muscle cells, and mediation of the immune response. The simplicity of the chemical structure of NO suggests it as a promising therapeutic agent, although biological effects may depend on concentration, receptor locations, and production sources.[11]

Furthermore, there are three isoforms of NO synthase, the enzyme that produces NO: neuronal, inducible (NOS2), and endothelial (NOS3).[11] The contribution of NO synthase on pulmonary hypertension in CDH remains controversial with some studies demonstrating a decrease in either NOS2 or NOS3 at birth, whereas others showed variable NOS2 expression depending on postnatal treatment and location of sampling.[12-14] Decreases in NOS3 expression in pulmonary arteries from human fetuses with CDH and pulmonary hypertension have also been reported.[13,14]

Our aim was to evaluate the changes due to TO and pulmonary ventilation on the production of NO through the expression of NOS3 and NOS2 in pulmonary vasculature in an experimental CDH rat model.

Materials and methods

Animals

Female Sprague–Dawley rats weighing around 250 g were subjected to mating during all days of the week. Couples were housed together overnight. The next day, the female genital region was examined for introital plugging. The presence of plugging was defined as gestational day 0. The study was approved by the Ethics Committee on Animal Experimentation of Ribeirao Preto Medical School–University of São Paulo # 043/2011.

Experimental groups

The fetuses were divided into eight groups ($n = 8$ per group): (1) control (C); (2) control ventilated (CV); (3) CDH (CDH); (4) CDH ventilated (CDHV); (5) TO control (performed with titanium clip); (6) TO ventilated; (7) TO + CDH (CDHTO); and (8) TO + CDH ventilated (CDHTOV).

Nitrofen administration

Pregnant rats were given 100 mg of nitrofen (2,4-dichloro-4-nitrodiphenyl ether, Maybridge; Tintagel, Cornwall, UK) dissolved in 1 mL of olive oil by gavage at gestational day 9.5 for inducing CDH, as described by Kluth *et al*.[15]

Tracheal occlusion

Rats were anesthetized at gestational day 18.5 with an intramuscular injection of ketamine 50 mg/mL (175 mg/kg) (Ketamina Agener, União Química Farmacêutica Nacional S/A, Embu-Guaçu, São Paulo, Brazil) associated with xylazine 10 mg/mL (2.5 mg/kg) (Dopaser, Laboratórios Calier S/A, Barcelona, Spain). After the peritoneal incision was made, a uterine horn was exposed to perform TO in two-three fetuses per uterus. A purse string suture with 6-0 polypropylene yarn (Prolene Ethicon, New Brunswick, NJ) was placed in the uterine wall. The head and neck of the fetus were exposed. The anterior aspect of the neck was dissected to expose the trachea. The titanium microclip (Teleflex Medical, Bannockbur, IL) was then placed with an appropriate clip applier. After TO, the externalized portion of the fetus (i.e., head and neck) was returned to the uterine cavity and the uterus was closed with the purse string suture previously performed.

Delivery and ventilation

At gestational day 21.5, pregnant female rats were anesthetized for harvesting using the same anesthetic protocol as the above procedure and the fetuses were collected through a median maternal laparotomy and hysterotomy. After weighing, the fetus was placed on a heating stage at 37°C and fixed with tape in the supine position. Then, a tracheal dissection was performed followed by tracheal section and intubation with a 24G Vialon (BD Insyte Autoguard, Becton Dickinson Infusion Therapy System Inc, Sandy, UT) catheter connected to time cycled ventilator (MiniVent type 845, Harvard Apparatus, Hugo Sachs Eletronik Harvard Apparatus GmbH, March, Hugstetten, Germany), with cycling frequency of 80 bpm, FiO_2 1.0, I:E ratio of 1:1, PEEP of 0 cmH_2O for 30 min. The CV fetuses were ventilated with a tidal volume of approximately 75 μL (13.5 mL/kg), and the TOV, CDHV, and CDHTOV fetuses were ventilated with 50 μL (9 mL/kg), based on our previous studies on lung and tidal volume.[16,17] Fetuses submitted to could have been ventilated with higher tidal volumes, but due to surgical manipulation, these fetuses were smaller in size, which