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Antenatal fetal VEGF therapy to promote pulmonary maturation in a preterm rabbit model

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ABSTRACT

Aim: To assess the effects of fetal tracheal administration of VEGF on pulmonary maturation in a preterm rabbit model.
Methods: On day 26 (term = 31 days), fetal rabbits received recombinant rat VEGF (30 μg in 70 μL normal saline) or placebo (normal saline 70 μL) intratracheally, with or without subsequent tracheal occlusion. Non-operated littermates served as internal controls. Fetuses were harvested on day 28 for morphometric study of the lungs or for mechanical ventilation and measurement of lung mechanics. In total, 96 fetuses from 42 does were used, 47 for ventilation and 49 for morphometry.
Results: In fetuses receiving intratracheal VEGF, an increase in immunoreactivity for Flk-1 was observed throughout the lung parenchyma. Tracheal occlusion (TO) adversely affected pulmonary mechanics as compared to un-occluded controls. That effect is partly reversed by intratracheal VEGF. Intratracheal injection of VEGF without tracheal occlusion improves lung mechanics but no more than what was observed in placebo injected controls.
Conclusion: Antenatal intratracheal VEGF administration was associated with an increase in Flk-1 immunoreactivity. It also improves lung mechanics, however more so when the trachea is occluded. Without TO, the effects were comparable to placebo controls.

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1. Introduction

In the USA, the incidence of very preterm birth is estimated at 1–2% of all live births, or between 40,000 and 80,000 infants based upon number of births in 2004 [1]. In Flanders, between 1991 and 2003 the overall incidence of prematurity increased by 0.23% per year. This increase is not fully explained by increases in twin pregnancies, advanced maternal age, assisted reproduction and obstetric intervention, which are all known contributors to preterm delivery [2]. Despite advances in neonatal intensive care and improved survival rates, lung immaturity and infant respiratory distress syndrome (IRDS) remain the leading causes of mortality in very immature infants. Antenatal interventions to accelerate lung maturation and in order to facilitate adaptation to preterm birth, consist currently in maternal administration of glucocorticoids (GC). Numerous trials have shown positive effects, including reduction in the incidence and severity of IRDS, air leaks, mortality rates and severe complications of prematurity like intraventricular hemorrhage [3,4]. However, antenatal GC do not reduce the incidence of chronic lung disease (CLD). On the contrary, younger and more immature infants survive at the expense of an increased incidence of CLD [5,6]. Therefore, there is a place for additional or alternative treatments to accelerate lung maturation or stimulate lung growth in the event of threatening preterm delivery, such that this would not lead to CLD.

In recent years, vascular endothelial growth factor (VEGF) has been postulated as a growth factor not only implied in angiogenesis and vasculogenesis, but which also plays an important role in normal lung development and the pathophysiology of IRDS and CLD [7]. Compernolle et al were the first to point to the possible therapeutic effect of exogenous VEGF administration in IRDS. They showed that prenatal (E17.5; canalicular stage) intra-amniotic administration as well as postnatal endotracheal administration of VEGF (0.5 μg) in preterm (E18.5; saccular stage, term = 20 days) mice resulted in clinical improvement of IRDS compared to control animals. On histology, VEGF-exposed animals displayed septal thinning and secondary crest formation [8]. Chen reported increased surfactant protein (SP) B and SP-D mRNA after intra-amniotic administration of recombinant VEGF (5 μg) in preterm rats

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(E18; term 22 days; canalicular stage) [9]. In summary, in rodents the exogenous administration of VEGF seems to enhance pulmonary maturation both structurally as well as surfactant production. Also in human fetal lung explants, mRNA levels of surfactant proteins SP-A and SP-C have been shown to be increased following addition of VEGF to the culture conditions [10]. This could not be reproduced in cultured isolated rat alveolar epithelial type II cells [11].

VEGF may have a protective role in CLD, a condition where both angiogenesis and alveologenesis are severely disturbed [7,12]. VEGF receptor blockade can mimic impaired angiogenesis and alveologenesis whereas postnatal adenovirus mediated VEGF gene therapy restores impaired alveolarization in hyperoxia-induced bronchopulmonary dysplasia in newborn rats [7,12,13]. In most physiological and pathological conditions, the specific stage of lung development or disease is critical for the observed effects of VEGF. This is a crucial issue when exogenous administration to fetuses or newborns is considered [14].

We aimed to study the effects of antenatal intratracheal VEGF administration using preterm fetal rabbits. In this model, a functional assessment of the pulmonary effects of antenatal therapy is feasible. We hypothesized that antenatal VEGF administration would stimulate type II cell differentiation, surfactant synthesis and release, which in turn would improve pulmonary mechanics.

2. Materials and methods

2.1. Animals and treatment groups

Time mated pregnant does (hybrid of Dendermonde and New-Zealand White) were obtained at day 23 of gestation from the animal farm of Leuven University. Prior to and after surgery, they were housed in separate cages at normal room temperature and normal daylight, with free access to water and chow. All animals were treated according to current guidelines on animal well-being. The Ethics Committee for Animal Experimentation of the Faculty of Medicine at the Catholic University Leuven approved the experiments. The Centre for Surgical Technologies and the involved investigators are accredited for experiments at Biosafety Level 1.

In total, fetuses from 42 does were randomly assigned to 4 treatment groups: intratracheal VEGF administration with (VEGF + TO) or without tracheal occlusion (VEGF-TO), intratracheal placebo administration with (placebo + TO) or without TO (placebo-TO). Non-operated littermates served as internal controls (CONTROL).

2.2. Antenatal intervention

The antenatal intervention took place on day 26 of gestation (canalicular stage, term = 31 days). Does were weighed and premedicated with ketamine 50 mg/kg kg (Ketamine 1000 CEVA®; CEVA Santé Animale, Brussels, Belgium), xylazine 6 mg/kg (Vexylan®; CEVA Santé Animale), and buprenorphine 0.03 mg/kg (Temgesic®; Schering-Plough), all injected intramuscularly. General anesthesia was maintained using a face mask with isoflurane 1.5% (Isoba® Vet; Abbott Laboratories Ltd., Queenborough, Kent, UK) in oxygen at 1 L/min. Maternal heart rate and oxygen saturation were monitored with a pulse oxymeter (Nellcor® N-20P; Nellcor Inc., Haasrode, Belgium). The doe was placed in the supine position, the abdominal wall was shaved and disinfected with povidone iodine (Isobetadine®; Asta Medica, Brussels, Belgium) and draped in a sterile fashion. Throughout all surgical procedures aseptic conditions were maintained.

Prior to opening of the abdominal wall, the subcutis was infiltrated with lidocain 2% (Xylocaine, Astra Zeneca) for additional analgesia. The pregnant bicornuate uterus was exposed through a low midline abdominal incision. After determining the fetal position by gentle palpation, a purse string (Prolene® 6-0, Ethicon, Dilbeek, Belgium) was placed and a 1 cm transversal linear incision was made on the anti-mesometrial side of the uterine wall. The fetal head and neck were exposed and supported by warm humidified swabs. An anterior midline incision was made in the neck and with careful dissection the trachea was exposed. A 3-0 polyester suture (Ticron®; Sherwood, Davis and Geck, Gosport, UK) was loosely tied around the trachea just cranial from the intended injection site. Thirty micrograms of recombinant rat VEGF164 in 70 µL vehicle or 70 µL normal saline (placebo) were injected intratracheally with a 300 µL syringe (Becton Dickinson Microfine Demi®, Becton Dickinson Inc, Sandy Utah, USA). As the needle was taken out, the ligature was tightened to prevent backflow. In the injection groups without TO, the needle was gently withdrawn after careful injection and the injection site was observed for leakage. The fetus was placed back in its gestational sack and the hysterotomy was closed in one layer with a running Prolene® 6-0 suture. A few milliliters of saline were added to the amniotic sac just before final closure, in order to replace the lost amniotic fluid. When litter size exceeded one, the two ovarian-end fetuses per doe were operated, one receiving VEGF, the other one placebo. The uterus was then returned into the abdominal cavity and the abdominal wall was closed in three layers using 3/0 polyglactin (Vicryl®, Ethicon, Dilbeek, Belgium) for the fascia and subcutis and 2/0 polyamide (Ethilon®) for the skin. Intramuscular medroxy progesterone acetate 4.5 mg (Depo-Provera®, Pharmacia Upjohn, Puurs, Belgium) was given postoperatively as a tocolytic, and 300,000 units of Penicillin G (Penicillin®, Continental Pharma, Brussels, Belgium) to prevent infection. After recovering in the operating facility, the animals were returned to their cages with free access to water and chow.

2.3. Delivery and harvesting of the fetuses

Fetuses were harvested by cesarean section for lung morphometry or ventilation and measurement of lung mechanics. Day 28 (saccular stage) was chosen as harvesting point because at that time fetal rabbit lungs are surfactant deficient and antenatal interventions that are expected to stimulate pulmonary maturation may show a measurable effect [15]. Does were premedicated and anesthetized as described above. The abdominal wall was opened and the uterus exposed. Fetuses were delivered one by one, the fetal condition was assessed and non-survivors were excluded from further analysis. After harvesting, the doe was euthanized with an IV bolus (1 mL/kg) of a mixture of embutramide 200 mg, mebezonium 50 mg and tetracain hydrochloride 5 mg (T61® Intervet Belgium NV, Mechelen, Belgium).

Fetuses were immediately weighed using a scale accurately up to 0.001 g (GF-200, DISI systems NV/SA, Wilrijk, Belgium). Fetuses that were assigned for ventilation, received 0.1–0.2 µg/g of xylazine intraperitoneally. Their trachea was exposed by an anterior midline incision and a tracheostomy was performed to advance an 18 gauge steel cannula, which was connected to the ventilator (Flexivent®, Scireq, Montreal, Canada). A polyglactin 3-0 (Vicryl®, Ethicon, Groot Bijgaarden, Belgium) suture was passed under and tied around the trachea, enveloping the cannula to create an airtight seal. Fetuses were ventilated one by one with a maximum of 3 per doe. Fetuses assigned for anatomical and morphometric study received an overdose of xylazine.

2.4. Anatomical findings

Fetuses were carefully dissected and the lungs were weighed. The lung to body weight ratio (LBWR) was calculated from the fetal body weight (FBW) and total lung weight. The right lung was immersion fixed in toto in 6% buffered formalin for 24 h and the left lung was snap frozen. The formalin fixed right lungs were embedded in paraffin and cut into 5 µm sections and stained with Hematoxylin Eosin and Elastica van Gieson (Hart's method) using Weigert's solution (resorcinol-fuchsin) [16]. These slides were used for airway and vascular morphometry. Lung sections

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