Pre-Clinical Large Animal Model of Thoracic Insufficiency using Yucatan Mini Pig

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INTRODUCTION: Thoracic Insufficiency Syndrome (TIS) represents a novel form of postnatal pulmonary hypoplasia and restrictive respiratory disease that occurs in children (<10 yrs.) with congenital or acquired anomalies of the spine and thorax [1]. These constrictive malformations of the thorax limit the space available for the lungs to grow and hinder pulmonary function, manifest clinically as: 1) hypoventilation (TCO₂) - the result of mechanically restricted chest wall and diaphragmatic motion; 2) impaired respiration (O₂) – the result of impaired lung development with retardation of alveolarization, maturational and increased septal wall thickness, which increase the alveolar-arterial gradient (A-a V) and a 1 L of O₂. We previously developed an ex vivo model of TIS by tethering the rib cage in very young rabbits to create a thoracoscopic scoliosis paraclinically evaluate the effect of thoracic deformity on the growth and development of the lung and impact on pulmonary function [2]. Similar to children, with increasing deformity there was a decreases in lung mass, and pulmonary function (functional residual capacity and forced vital capacity). However, the diminutive size of the rabbit led to a premature evaluation of clinically relevant implants and surgical interventions to mediate the life threatening effects of TIS, diminishing its translational value. The purpose of this study was to develop a pre-clinical mini pig model of TIS induced by tethering the rib cage, similar to the rabbit model.

METHODS: Under institutional approval two 8-week-old Yucatan mini-pigs weighing 8 kg were used. Each pig was placed in left lateral recumbency. A longitudinal incision was made along the dorsal aspect of the ribs 5-12 to expose the ribs without disrupting the parietal pleura. The ribs were divided into three groups (cranial, mid, caudal) and tied together with fiberwire (Fig. 1). The incision was closed in layers. Pigs recovered in a high oxygen environment, monitored closely by veterinary staff, maintained on an extensive analgesic regime for the first 72 hours. Imaging: Pigs were anesthetized for serial CT scans (Omnitom® 8-slice small-bore mobile CT): pre-operative, every other week to 32 weeks postoperatively, then at 37, 44, 52, 64, and 68 weeks postoperatively to document progressive thoracic deformity and corresponding lung hypoplasia. The extent of scoliosis was measured as the Cobb angle on the coronal plane CT reconstruction (θ) and the extent of kyphosis as the Cobb angle on the sagittal plane CT reconstruction (θk). The mean lung volume (MLV) was calculated from segmented CT images using standardized thresholds to differentiate aerated lung tissue sequentially from 2 to 68 weeks (Fig. 2) [3]. Pulmonary Function Following endotracheal intubation, animals were ventilated under volume-controlled ventilation mode, with inspiratory-to-expiratory ratio of 1:2, tidal volume of 10 mL/kg, respiratory rate adjusted to maintain an end-tidal CO₂ tension of 35-40 mmHg.

Airway pressures were measured using a Pitot-based flow meter connected between the orotracheal tube and the Y-piece of the breathing system. Dynamic lung compliance, Cdyn, was calculated from the slope of (AV(ΔP)/e) of the inspiratory mechanical volume flow loops.

RESULTS: Tethering ribs comprising the right hemithorax induced a convex kyphoscoliosis (θ=27.5°; θk=17.8°), with progression of the kyphotic deformity (θ=25.0°; θk=38.4°) at 68 weeks. 3D lung renderings revealed differences in morphology at 2 weeks for TA compared to AMC (Fig 2A, B, E). Thus, by 20 weeks, the lung morphology of the TA had corrected (Fig 2C, F), and by 68 weeks, the lung morphologies of TA vs. AMC were similar (Fig 2D, G). MLV steadily progressed over time with thoracic growth. There was a notable difference in MLV of the TA when compared to the AMC over weeks 2 to 26; however starting at week 22, the MLV of the TA was equivalent to the AMC. At 68-weeks, MLV for AMC =1523.3 cm³ vs. TA =1454.0 cm³ (Fig. 2H). Tethering rib most affected respiratory mechanics; at 2 weeks, Cdyn for TA was similar to AMC (29 vs. 25 mL/cmH₂O, respectively), but at 6 weeks respiratory compliance diminished for TA relative to AMC (Cdyn=32 vs. 39 mL/cmH₂O, respectively), and remained unchanged at ~32 mL/cmH₂O through 68 weeks, whereas AMC Cdyn continued to progress to ~48 mL/cmH₂O at 20 weeks, then plateaued through 68-weeks (Fig 2J).

Thus despite normalization of MLV, there was a loss of compliance as a result of a constricted (stiff) chest wall induced by tethering. Intermittent positive pressure ventilation (IPPV), used clinically to expand the lungs, increased Cdyn by more than 65% for AMC at 68 weeks, while IPPV increased Cdyn for TA by only 15% at 68 weeks. In contrast flow-controlled ventilation (FLEX) increased Cdyn for AMC from 28 to 62 mL/cmH₂O at 68 weeks, but also increased Cdyn for TA from 28 to 58 mL/cmH₂O. (Fig. 2I). DISCUSSION: Similar to children with TIS, despite normalization of lung volume and morphology, the result of compensatory hypertrophy of the unsupported lung, constraining the chest wall by tethering the ribs decreased respiratory compliance in the Yucatan mini-pig model. Non-surgical treatment of TIS often requires nocturnal positive pressure ventilation, and the reduced respiratory compliance affected IPPV more than FLEX. Conventional volume-controlled ventilation is achieved by active inflation of the lungs followed by passive (elastic recoil) emptying during expiration. In contrast, FLEX ventilation is a procedure that modulates the passive expiratory phase. Reducing the initial high-expiratory peak flow to a more linear flow potentiates and prolongs the expiratory flow phase. Thus FLEX results in “energy preservation” during expiration. As such FLEX appears to be better compensate for decreased respiratory compliance than IPPV by stabilizing dependent areas of the lung and preventing premature bronchial tree collapse during inspiration. The exact mechanism of how this is achieved requires further investigation, including differentiating chest wall compliance from lung parenchyma tissue compliance and histological evaluation of the bronchial and alveolar morphology. CLINICAL RELEVANCE/SIGNIFICANCE: Developing a large pre-clinical animal model for TIS as a testing platform to evaluate normal respiratory development, the pathoanatomy associated with thoracic insufficiency and to parametrically evaluate the efficacy of different treatment strategies to ameliorate TIS is fundamental to reducing the morbidity and mortality of TIS.


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