Pulmonary Pharmacology & Therapeutics 30 (2015) 22–31

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

Plp1 gene duplication inhibits airway responsiveness and induces lung inflammation

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ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form
26 September 2014
Accepted 25 October 2014
Available online 4 November 2014

Keywords:
Pelizaeus-Merzbacher disease
Airway pharmacology
Lung mechanics
Phase angle

Chemical compounds studied in this article:
Methacholine chloride (PubChem CID: 6114)
Albuterol sulfate (PubChem CID: 39859)

ABSTRACT

Mice with Plp1 gene duplication model the most common form of Pelizaeus-Merzbacher disease (PMD), a CNS disease in which patients may suffer respiratory complications. We hypothesized that affected mice would lack airway responsiveness compared to wild-type and carrier mice during methacholine challenge. Wild-type ($n = 10$), carrier female ($n = 6$) and affected male ($n = 8$) mice were anesthetized-paralyzed, tracheostomized and ventilated. Respiratory mechanics were recorded at baseline and during escalating doses of nebulized methacholine followed by albuterol. Lung resistance (R_L) was the primary endpoint. Lung tissues were assayed for inflammatory and histological differences. At baseline, phase angles were higher in carrier and affected mice than wild-type. Dose-response R_L curves in affected and carrier mice indicated a lack of methacholine response. Albuterol reduced R_L in wild-type and carrier, but not affected mice. Affected mice exhibited lower interleukin (IL)-6 tissue levels and alveolar inflammatory infiltrates. Affected and carrier mice, compared to wild-type, lacked airway reactivity during methacholine challenge, but only affected mice exhibited decreased lung tissue levels of IL-6 and inflammation.

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1. Introduction

Pelizaeus-Merzbacher disease (PMD), an X-linked neurodegenerative disease characterized by dysmyelinating (improper myelin formation) and demyelinating (degradation of myelin) processes in the central nervous system (CNS). PMD [MIM#312080] is caused by mutations of the proteolipid protein 1 gene [*PLP1*; MIM#300401] that encodes proteolipid protein (PLP), the most abundant protein in CNS myelin [1,2]. PLP was shown to be involved in compaction of myelin and in the formation and signaling of integrin receptor complexes in in-vitro animal cell studies [3–5]. Patients with PMD usually present with nystagmus, hypotonia, and titubations at birth or in early childhood, slowly progressing to ataxia and spasticity later in life, and hypomyelination is observed on magnetic resonance imaging (MRI) of the CNS [6]. Heterozygous female carriers are not usually PMD affected/symptomatic [7]. The estimated prevalence of PMD in the United States is 1:200,000 to 1:500,000 [6]. The mortality rate (per million individuals per year) for ages <5 and ≥5 years is estimated to be 0.037 and 0.033, respectively, and death ranges from 4 to 66 years [8].

PMD can be caused by mutations that affect the coding region and splicing of *PLP1*; however, the most common mutation is the duplication of a genomic region that includes the *PLP1* gene [9]. Duplication of *PLP1*, which accounts for approximately 62% of PMD

Abbreviations: CNS, central nervous system; Cdyn, dynamic lung compliance; C_{st}, static lung compliance; IL, interleukin; LBI, labored breathing index; PMD, Pelizaeus-Merzbacher disease; PLP, proteolipid protein; *PLP1*, human proteolipid protein 1 gene; *Plp1*, mouse proteolipid protein 1 gene; *Plp1*dup, name of the mouse model that has a genomic duplication that includes *Plp1*; PhRTB, phase relation during total breath; PNS, parasympathetic nervous system; R_L, lung resistance; SNS, sympathetic nervous system; TNF-α, tumor necrosis factor−α.

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http://dx.doi.org/10.1016/j.pupt.2014.10.004
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cases when strict clinical criteria are applied [10], results in the classical form of disease that is typically in the middle of the clinical severity spectrum for PMD. Duplication may lead to overexpression of PLP, which has been shown to cause both brain inflammation and abnormal trafficking of PLP to the cell surface of oligodendrocytes, suggesting mechanisms for disease pathogenesis [11,12]. There are no therapeutic options available for PMD, but compounds that lower the expression of *PLP1* may provide a rational treatment for PMD that is due to an extra copy of the gene.

Although PMD is a neurological disease of the white matter of the CNS in origin, it affects many other organ systems including the respiratory system, so a multidisciplinary approach is recommended for optimal care [6]. Airway aspiration and/or severe scoliosis are respiratory complications, which are major causes of morbidity and mortality [6,13]. The severe or connatal/congenital form of PMD features respiratory distress, stridor and pharyngeal weakness [14].

In this study, we tested whether PMD due to genomic duplication is associated with disruption of respiratory autonomic homeostasis. We used a mouse model, called *Plp1*dup, that has a genomic duplication that includes *Plp1* and is similar in size and structure to most human PMD duplications. These mice develop neurological sequelae that model human PMD [15], but a respiratory phenotype has not been described in this *Plp1*dup model. We used methacholine as a drug challenge to the respiratory control system in the mouse model. Methacholine is a cholinergic synthetic analog of acetylcholine (ACh), an important neurotransmitter in both the CNS and the peripheral nervous system that produces smooth muscle contractions of the airways through muscarinic M3 receptors located in the tracheobronchial tree and in different regions of the brain [16]. The aims of this translational study were to compare respiratory mechanics under rest, during methacholine challenge, and after β_2-adrenoceptor agonist exposure to determine biomarkers of lung inflammation, and to examine structural lung tissue differences among wild-type, carrier, and *Plp1*dup affected mice. Our translational studies on *Plp1*dup mice are ongoing with the focused goal of identifying the respiratory phenotype of the disease, the mechanisms of disease pathogenesis, and identification and testing of therapeutic interventions.

2. Methods

2.1. Animal model

Generation of the *Plp1*dup murine model of PMD using Mutagenic Insertion and Chromosome Engineering Resources (MICER) and characterization of the neurological phenotype are as previously described [15,17]. *Plp1*dup mice are an appropriate model for our studies because they have a genomic duplication on the X-chromosome that includes *Plp1* and is similar in size and structure to the duplications detected in human PMD patients and because the neurological phenotype is similar to that in human patients [15,18,19].

2.2. Animal preparation

This study of methacholine challenge was approved by the Nemours Institutional Animal Care and Use Committee, Department of Nemours Biomedical Research, in accordance with the National Institutes of Health guidelines. Three groups, wild-type mice ($n = 10$; five females and five males), carrier mice ($n = 6$; all females), and *Plp1*dup affected mice [15] ($n = 8$; all males), were studied at 6 months of age -equivalent to 20–30 years old in human age- [20]. All mice were on a B6.129 mixed background. Animals were anaesthetized with a ketamine-xylazine cocktail (50 mg/mL:10 mg/mL, 1:1 ratio) by intraperitoneal (IP) injections at a maximal dose of 0.08 mL/10 g (range 0.04–0.08 mL/10 g). Oximetry, heart rate, percentage of tail perfusion were monitored using a pulse oximeter (MouseSTAT, Smiths Medical, Waukesha, WI). Body temperature was monitored using a laser thermometer (ThermoWorks, Alpine, UT) and maintained using a far infrared warming pad (Kent Scientific Corporation, Torrington, CT).

2.3. Respiratory inductance plethysmography

Before invasive procedures in the unconscious animals were performed, respiratory inductance plethysmography was performed to measure thoracoabdominal motion indices of asynchronic breathing patterns (phase angle and phase relation during total breath [PhRTB] and labored breathing index [LBI]). These measurements are not invasive as compared with conventional measurements of resistance and compliance in unconscious mice, where direct measurements of the airway flow at the tracheal opening are required. Measurements were performed using inductive bands (RespiRod bands, Wilmington, DE) designed by the first author and collaborators at the Nemours Pediatric Engineering Research Laboratory (Fig. 1).

These bands were designed for use with a snap connector and cloth fastener for use with a RespiTrace device, the SomnoStarPT Unit (Sensormedics, Yorba Linda, CA), and in mice ≥28 g. Preliminary testing was performed to assure correct operation, which consisted of observation of the Lissajours and Konno-Mead loops at different respiratory frequencies and positive end-expiratory pressures. After ensuring that the animal skin was dry and free of oil or other substances, the animal was placed on top of the two bands; the top band (RC) was positioned under the armpit of the upper limb and the bottom band (AB) at the largest abdominal circumference parallel to the RC band.

The respiratory inductance plethysmography method uses sinusoidal coils of wire sewn into elastic cloth bands. Co Flex NL (Andover, Salisbury, MA) elastic bands were used, which were identical -with respect to material properties-to the materials used in the Respiratory Plus bands (RespiBands Plus; VIASYS Respiratory Care, Yorba Linda, CA) approved for clinical use. The elastic material was shortened to 1.5-cm wide and 1-mm thick. This elastic bandage works effectively since it binds to itself and maintains elasticity when compressed. The original amplitude and frequency of the field-wire waveform in the Respiratory Plus band was not sensitive enough to pick up the diminutive movements of the rodent, so we developed the RespiRod bands as follows. We designed a custom

Fig. 1. RespiRod bands. The minimum total stroke volume that these bands can detect is 3 cc at a respiratory frequency of 120 breaths per minute, with a minimum positive end-expiratory pressure of 3 cm H_2O.

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