EFFECT OF TIRASEMIV ON SUBMAXIMAL RODENT DIAPHRAGM STRENGTH AND RESPIRATORY FUNCTION
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ABSTRACT

Background: Diaphragm weakness, which is characterized by significant losses in function, is a primary component of the pathophysiological changes that lead to respiratory failure. Tirasemtiv is a fast skeletal troponin activator, previously shown to increase submaximal force in rat and human lower leg muscles.

Objective: The objective of this study was to characterize the effect of tirasemtiv on calcium sensitivity and force production ex vivo in rat and mice diaphragm muscle.

Methods: For in vitro skinned (permeabilized) fiber studies, Sprague Dawley rat diaphragm muscles were rapidly dissected, rinsed in physiological saline, and then incubated in skinnning and storage solution. Single muscle fibers were dissected from larger segments of tissue in rigor buffer. The fibers were then suspended between a force transducer and a fixed post. The muscle force-calcium (pCa) relationship in diaphragm muscle was investigated in single rat diaphragm fibers treated with either 1% DMSO (vehicle treatment), or tirasemtiv (0.1µM, 1µM, or 10µM) over -log(10) calcium concentrations (pCa) ranging from 8 to 4.

For intact diaphragm muscle, contractile force was measured by electrical field stimulation in an organ bath system. The diaphragm and the last floating rib from B6SJL mice were excised, rinsed in physiological saline, placed in a temperature controlled water-jacketed chamber containing Krebs-Henseleit buffer. Braided silk sutures were tied at the central tendon and floating rib and attached to a force transducer between two platinum electrodes. The force-frequency profile of the muscle was obtained by stimulating the muscle at frequencies between 5-150 Hz. Tirasemtiv (1 µM in DMSO) was directly added into the bath.

Results: Tirasemtiv increased the calcium sensitivity of rat diaphragm muscle, shifting the force-pCa relationship of skinned fibers in a dose-dependent manner. Compared to DMSO-treated skinned diaphragm muscle fibers, 10µM tirasemtiv increased the pCa at 50% maximum tension (pCa50) 10-fold (vehicle: -log(10) calcium concentrations (pCa) 7.51 ± 0.05, n=5/group). In intact muscle, at submaximal stimulation frequencies less than 200Hz, tirasemtiv (1µM) increased mouse diaphragm tension ex vivo compared to vehicle-treated diaphragm strips (vehicle n=11, tirasemtiv n=5, p<0.05 at 5, 10, and 20 Hz).

Discussion and conclusions: Pathological conditions that lead to diaphragm weakness can have severe consequences, ranging from dyspnea and reduced quality of life to respiratory failure and death. The fast skeletal troponin activator, tirasemtiv, has previously been shown to increase force at submaximal rates of nerve stimulation rat and human lower leg muscles. These results suggest that tirasemtiv and other fast skeletal muscle troponin activators may be viable therapeutics for improving respiratory muscle function.

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INTRODUCTION

• Diaphragm weakness is a primary component of the pathophysiological changes that lead to respiratory failure.
• About half of the diaphragm is composed of Type II fast skeletal muscle fibers (Humans: 50% Type II; Rodents: 60% Type II).
• Tirasemtiv is a fast skeletal troponin activator that increases sarcomere calcium sensitivity, force production, and respiratory function in rat and mouse diaphragm muscle.

METHODS

In vitro muscle force-Ca²⁺ relationship: Sprague Dawley rat diaphragm muscles were rapidly dissected, rinsed in physiological saline, and then incubated in skinnning and storage membrane permeabilization solution. Single muscle fibers were dissected from larger segments of tissue in rigor buffer. The fibers were then suspended between a force transducer and a fixed post. The muscle force-calcium (pCa) relationship in diaphragm muscle was investigated in single rat diaphragm fibers treated with either 1% DMSO (vehicle treatment), or tirasemtiv (0.1µM, 1µM, or 10µM) over -log(10) calcium concentrations (pCa) ranging from 8 to 4.

Ex vivo diaphragm force: Diaphragm isometric force was measured by electrical field stimulation in an organ bath system. The diaphragm and the last floating rib from B6SJL mice were excised, rinsed in physiological saline, placed in a temperature controlled water-jacketed chamber containing Krebs-Henseleit buffer. Braided silk sutures were tied at the central tendon and floating rib and attached to a force transducer between two platinum electrodes. The force-frequency profile of the muscle was obtained by stimulating the muscle at frequencies between 5-150 Hz. Tirasemtiv (1 µM in DMSO) was directly added into the bath.

In vivo respiratory function: B6SJL-SOD1G93A mice were orally dosed with vehicle or 10 mg/kg tirasemtiv in a blinded, cross-over design. After oral treatment, mice were placed in unrestrained whole body plethysmography chambers for 30 minutes of acclimation. After acclimation, respiratory parameters, including tidal volume, respiratory rate, and minute ventilation, were monitored for 10 minutes at room air.

RESULTS

• Tirasemtiv increases diaphragm force at submaximal nerve stimulation.
• Tirasemtiv increases diaphragm force in response to submaximal rates of nerve stimulation.
• Tidal volume in vivo in a mouse model of ALS.

CONCLUSIONS

• Pathological conditions that lead to diaphragm weakness can have severe consequences, ranging from dyspnea and reduced quality of life to respiratory failure and death.
• The fast skeletal troponin activator tirasemtiv increased:
  - Rat diaphragm fiber Ca²⁺ sensitivity in a concentration-dependent manner.
  - Mouse diaphragm submaximal force production ex vivo.
  - Tidal volume in vivo in a mouse model of ALS.
• These results suggest that tirasemtiv and other fast skeletal muscle troponin activators may be viable therapeutics for improving respiratory muscle function.