The Direct Smooth Muscle Myosin Inhibitor, CK-2018571, Represents a Novel Therapeutic Approach for Bronchodilation

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INTRODUCTION

Smooth muscle myosin is a mechanochemical enzyme that hydrolyzes ATP to generate mechanical force; ultimately all signaling pathways that modulate smooth muscle tone converge upon the regulation of this motor protein. Using high throughput screening, we identified and subsequently optimized a class of selective inhibitors of smooth muscle myosin. Previously we showed that CK-2018509, a novel, potent, and selective inhibitor of the enzymatic activity of smooth muscle myosin, decreased the mean arterial pressure in two animal models of hypertension [1]. Another novel smooth muscle myosin inhibitor, CK-2019165, an active pro-drug of CK-2018509, was previously shown to decrease right ventricular systolic pressure (RVP) in two rat models of pulmonary arterial hypertension [2]. Given its central role in generating smooth muscle contractility in the settings of chronic obstructive pulmonary disease (COPD) and asthma, direct inhibition of smooth muscle myosin could provide a novel and effective means to reduce bronchoconstriction in asthma and COPD. The objective of the present study was to evaluate the pharmacology of CK-2018571 and CK-2019165 in rat models of bronchoconstriction.

METHODS

Biochemical Assays: Assays were performed in low salt PM12 buffer (12 mM K2PO4, 20 mM Hepes, pH 6.8) in the presence of actin and 250 μM ATP (r-10-fold above the Km). Actin ATPase rates were normalized using equations containing an apparent amount of DNM5. Myosin binding was measured by depletion of soluble myosin from binding reactions using smooth muscle myosin 51 fragment (chicken), and 5 μM bovine cardiac and ADP were included at 1 mM where indicated. Reactions were allowed to equilibrate for 15 minutes prior to centrifugation (5400 x g, 30 minutes), however ATP was added just prior to centrifugation to minimize hydrolysis. Supernatants were analyzed by SDS-PAGE following by staining with Coomassie brilliant blue.

Skinned Ring Assay: Endothelium-denuded rat tail artery segments were cut into 3 mm helical rings, mounted on an isometric force transducer with a resting tension of 0.5 g and incubated for 30 minutes at room temperature in normal H-T buffer. Tissues were incubated with skinnning solution containing 1% Triton X-100 for 1 hour at room temperature. CK-2018571 was added to the tissue for 15 minutes, followed by addition of solutions with increasing calcium. Force was recorded at each calcium concentration. Data were presented as a percent change from the baseline values.

Tracheal Ring Assay: The trachea was removed and placed in Krebs-Henseleit buffer aerated with 95% O2 and 5% CO2. The trachea was cut into 2 mm rings, mounted on a tissue bath apparatus and maintained at a baseline tension of 2 g. CK-2018571-induced relaxation was recorded in preparations pre-contracted with a sub-maximal concentration of methacholine (3 μM).

Throphosphorylation: Triton-permeabilized preparations were incubated in rigor solution containing ATPs (1 mM) for 10 minutes. CK-2018571 was added 15 minutes before addition of the ATP. ATP-induced contraction was measured for 60 minutes and the relaxation was expressed as the percentage of the maximum force.

In vivo Assay: Anesthetized Rodent Model of Airway Resistance & Compliance Male Sprague-Dawley rats were anesthetized with Ketamine/Xylazine/acepromazine (80/101/0 mg/kg) cocktail and tracheotomized with a 14 g tracheal cannula. Rats were paralyzed with Pancuronium Bromide at 2 mg/kg, i.e. to prevent spontaneous breathing. Immediately rats were placed on a Resistance & Compliance Plethysmograph (Buxco Research Systems). Once rats were stabilized and a baseline was collected, CK-2019165 was intra-tracheally nebulized via an Aeroneb Lab micropump nebulizer. Five minutes later, dose-dependent bronchodilatation to methacholine was measured.

Conscious Rodent Model of Unrestricted Whole Body Plethysmography Male Sprague-Dawley rats were dosed with CK-2019165 via conscious spontaneous inhalation of aerosolized solution in an in-house custom built 12-slot closed circuit chamber using a PAR Li Pico Jet Nebulizer (2290, PARI Respiratory Equipment), pressurized to 22 psi with a carrier gas mixture of 21% O2, 5% CO2, 74% N2 with a flow rate of 12 L/min. After dosing, rats were placed in an Unrestricted Whole Body Plethysmograph (Buxco Research Systems), and animals were allowed to acclimate. Baseline measurement was collected and rats were subsequently challenged with nebulized methacholine (100 μg/μL of 20 mg/mL).

RESULTS

Figure 1: CK-2018571 selectively inhibits the ATPase activity of smooth muscle myosin

Four competitive inhibitors of myosin ATPase have been identified and subsequently optimized. Inhibition of myosin ATPase activity is accompanied by a significant decrease in the activity of smooth muscle myosin 51 fragment. However, ATP-inhibited CK-2018571 did not significantly decrease myosin ATPase activity to the same extent as ATP-inhibited CK-2018509 (Figure 1).

Figure 2: CK-2018571 does not promote strong actomyosin binding

Accurate binding of soluble smooth muscle myosin (skinned 51 fragment) is critical for the presence of active and correctable smooth muscle myosin. Electrophoresis activity of smooth muscle myosin over other myosin isoforms would indicate weak and strong actin binding in a weak actin-binding state. CK-2019165 inhibited the methacholine-induced bronchodilatation in anesthetized, paralyzed and mechanically ventilated resistance and compliance rat and unanesthetized rats (Figure 2).

Figure 3: CK-2018571 inhibits calcium-dependent contraction of skinned cardiac artery smooth muscle preparation

Figure 4: CK-2018571 causes concentration dependent relaxation of isolated tracheal rings

In vitro CF: CK-2018509 inhibited sinusoidal contraction in isolated tracheal rings in a concentration dependent manner with an EC50 of 0.15 μM and Emax of 100%. Symbols are mean ± standard error values.

Figure 5: CK-2018571 relaxes the ATP-induced contraction in ATnP6 treated caudal artery

Effects of ATP in the presence or absence of CK-2018571 (P < 0.05) in isolated skinned and triphosphorylated smooth muscle preparations. Symbols are mean ± standard error values.

Figure 6: Effect of CK-2018571 on methacholine-induced bronchodilatation in anesthetized restrained and non-restrained rat and unanesthetized rat models

Effects of CK-2018571 on methacholine-induced bronchodilatation in anesthetized restrained and non-restrained rat and unanesthetized rat models are shown. Symbols are mean ± standard error values.

Figure 7: Effect of CK-2018571 on methacholine-induced bronchodilatation in unrestrained whole body plethysmography rat model

Effect of CK-2018571 on methacholine-induced bronchodilatation in unrestrained whole body plethysmography rat models are shown. Symbols are mean ± standard error values.

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3. CK-2018571 inhibits smooth muscle myosin in a weak actin-binding state.

4. CK-2018571 inhibits calcium-induced force development in skinned cardiac smooth muscle and relaxes skinned rings activated by both smooth muscle myosin and non-muscle myosin. The lack of a direct inhibitor of smooth muscle myosin.

5. CK-2018571 relaxes methacholine pre-

6. The data presented suggest that direct inhibition of smooth muscle myosin could be a novel therapeutic strategy for the treatment of chronic obstructive pulmonary disease and asthma.

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