A DIRECT INHIBITOR OF SMOOTH MUSCLE MYOSIN AS A NOVEL THERAPEUTIC APPROACH FOR THE TREATMENT OF SYSTEMIC HYPERTENSION
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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 on the regulation of this motor protein. While many drug mechanisms that relax smooth muscle reduce blood pressure, blood pressure still remains inadequately treated in many patients. Given its central role in generating smooth muscle contractility, direct inhibition of smooth muscle myosin should provide a novel and effective means to reduce blood pressure. Using high throughput screening, we identified and subsequently optimized a class of selective inhibitors of smooth muscle myosin. CK-2018509 is a novel, potent, and selective inhibitor of the enzymatic activity of smooth muscle myosin. In addition to the biochemical mechanism of action, we further characterize here its pharmacology in skinned and native isolated blood vessels as well as in rodent models of systemic hypertension.

Methods

Biochemical Assays: Assays were performed in low salt PM12 buffer (12 mM K-Pipes, 2 mM MgCl2, pH 6.8) in the presence of actin and 250 µM ATP (5×10-fold above the K₅₀). Hydrolysis rates were normalized using reactions containing an equivalent amount of DMSO.

Myosin binding was measured by depletion of soluble myosin from binding reactions using smooth muscle myosin 51 fragment (human, recombinant) and 5 µM bovine cardiac actin. ATP and ADP were included at 1 mM where indicated. Reactions were allowed to equilibrate for 15 minutes prior to centrifugation (5400 g, 30 minutes), and ATP was added just prior to centrifugation to minimize hydrolysis. Supernatants were analyzed by SDS-PAGE followed by staining with Coomassie brilliant blue.

Skinned Ring Assay: Endothelium-denuded rat tail artery segments were cut into 4 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 skinned by incubation with solution containing 1% Triton X-100 for 1 hour at room temperature. CK-2018509 was added to the tissue for 15 minutes, followed by addition of solution with increasing calcium. Force generated at the plateau of each corresponding pCa was recorded. Data were presented as a percent change from the baseline values (Wilson et al., 2002).

Aortic Ring Assay: The thoracic aorta was removed and placed in Krebs-Henseleit buffer aerated with 95% O₂ and 5% CO₂. Arteries were cut into 2-mm rings, mounted on a tissue bath apparatus, and maintained at a baseline tension of 2 g. Endothelium-independent relaxation to CK-2018509 was recorded in preparations pre-contracted with a sub-maximal concentration of phenylephrine (EC₅₀ ~ 0.1 µM).

Throphosphorylation Assay: Triton-permeabilized preparations were incubated in rigor solution containing ATP(6 mM) for 10 minutes. CK-2018509 was added (1 mM) for 15 minutes before addition of ATP. ATP-induced contraction was measured for 60 minutes and the relaxation was expressed as percentage of the maximum force.

In vivo Assay: Spontaneously hypertensive rats (SHR) and Dahl salt-sensitive rats were purchased from Charles River Laboratory. Dahl salt-sensitive rats were fed 8% NaCl in their diet to maintain hypertension. Rats were placed in restrainers for blood pressure monitoring; blood pressure was measured via the carotid artery using a fluid-filled dome pressure transducer.

Results

Figure 1: Aortic ring assay with smooth muscle myosin (10 µM) are indicated by the 30% ADP and 40% ATP relaxation. Force development to ATP in the presence or absence of CK-2018509 shows complete inhibition at 100 nM, 50 µM ATP ADP ATP. Representative curves from duplicate reactions are shown in panels (A) and (B).

Figure 2: Skinned aortic rings from Sprague Dawley rats (n=3, mean ± sem). CK-2018509 inhibits calcium-induced contraction of skinned caudal ring preparation.

Figure 3: Smooth actomyosin binding is revealed by depletion of soluble smooth muscle myosin (recombinant human S1) upon binding reaction supernatants (free fraction) Loading Controls DMSO (1%) CK-2018509 (100 µM) ATP ADP ATP.

Figure 4: Biological effect of CK-2018509 on mean arterial pressure (MAP) in spontaneously hypertensive rats (n=4). We show a significant decrease in MAP with an IC50 of 4.6 ± 0.5 µM.

Figure 5: Effect of CK-2018509 on mean arterial pressure (MAP) and heart rate (HR) in spontaneously hypertensive rats (n=4). We show a significant decrease in MAP and HR with an IC50 of 4.6 ± 0.5 µM.

Figure 6: Concentration response curves of CK-2018509 in isolated aortic rings from Sprague-Dawley rats (n=6, mean ± SEM). CK-2018509 inhibits calcium-induced force development of the dorsal caudal ring with competitive inhibition observed at 1 µM ATP. Data were presented as a relative change from control values (Wilson et al., 2002).

Figure 7: Effect of CK-2018509 on mean arterial pressure (MAP) and heart rate (HR) in Dahl salt-sensitive rats (n=4) compared with vehicle.

Conclusions

1. CK-2018509 selectively inhibits the ATPase activity of smooth muscle myosin as compared to either myosin II isoforms (non-muscle myosin, cardiac and skeletal muscle myosins).

2. CK-2018509 confers its vasorelaxation activity by binding to smooth muscle myosin in a weak acto-myosin binding state.

3. CK-2018509 inhibits calcium-induced force development in skinned caudal arteries and relaxes skinned cells activated by thromboxane A₂, consistent with relaxation occurring as a consequence of direct inhibition of smooth muscle myosin.

4. CK-2018509 relaxes phenylephrine pre-contracted aortic rings in a concentration-dependent manner, suggesting its potential use as a vasodilator.

5. CK-2018509 decreases the elevated mean arterial blood pressure with minimal effect on heart rate in two animal models of hypertension: spontaneously hypertensive rats and Dahl salt-sensitive rats.

Taken together, these data suggest that direct inhibition of smooth muscle myosin may be a novel therapeutic approach for the treatment of systemic hypertension.

References