

Inhibition of Smooth Muscle Myosin, a Novel Therapeutic Approach for Pulmonary Hypertension

David Ho, Xin Zhao, Li Chen, You-Tang Shen, Xiangping Qian, David J. Morgans, Stephen F. Vatner, Fady I. Malik

CV Dynamics, Inc. and Dept. Cell Biology and Molecular Medicine and Cardiovascular Research Institute, University of Medicine and Dentistry of New Jersey; Newark, NJ.; Cytokinetics, Inc. South San Francisco, CA

INTRODUCTION

In contrast to the therapy of systemic hypertension, there are relatively few approaches available to treat pulmonary hypertension. We had previously in a dog model of systemic hypertension demonstrated that intravenous administration of smooth muscle myosin inhibitors produced potent systemic vasodilatation. The goal of the present study was to examine the extent to which CK-2019165 (CK-165), a smooth muscle myosin inhibitor exemplifying this new approach, ameliorated pulmonary hypertension in a pig model. The pig model exhibits more robust pulmonary vasoconstriction than other species and the level of hypoxia-induced pulmonary hypertension is similar to those observed in patients.

GOAL

To establish a porcine model of acute pulmonary arterial hypertension, by either hypoxia or administration of a thromboxane analog and to examine the extent to which the inhibition of smooth muscle myosin by CK-2019165 (CK-165) administered either intravenously or by inhalation is able to ameliorate pulmonary hypertension in chronically instrumented pigs.

METHODS

- Six domestic female pigs were surgically instrumented with a Transonic flow probe around the main pulmonary artery and catheters placed in the pulmonary artery, descending aorta, and right and left atria to measure systemic and pulmonary vascular dynamics.
- One week after surgery, pulmonary hypertension was induced by hypoxia, i.e., intubated and breathing at reduced FIO₂ (0.10), or infusion of the thromboxane analog (U-46619, 0.1 μg/kg/min, i.v.).
- CK-165 (4 mg/kg) or sodium nitroprusside (2 mg/kg) (SNP) was administered intravenously, or a volume of 2-3 ml of 10 mg/ml (CK-165) or 4 mg/ml (SNP) was nebulized over 4-5 min down the endotracheal tube using a Pari LC Plus compressed air nebulizer. The nebulizer was operated at 20 psi which results in an output of ~4.5 L/min.
- Arterial SPO₂ was monitored continuously using a pulse oximeter during the entire experimental period.

1st Hypothesis

A smooth muscle myosin inhibitor will ameliorate hypoxia induced pulmonary hypertension

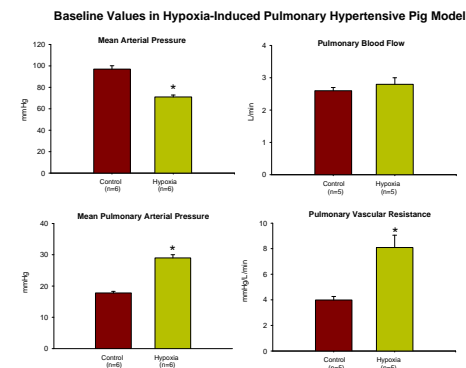


Fig 1: Baseline values of mean arterial pressure, mean pulmonary arterial pressure, pulmonary blood flow and pulmonary vascular resistance in the hypoxic condition compared to control. Mean arterial pressure, pulmonary arterial pressure and pulmonary vascular resistance were significantly increased in the hypertensive condition compared to control. *P<0.01

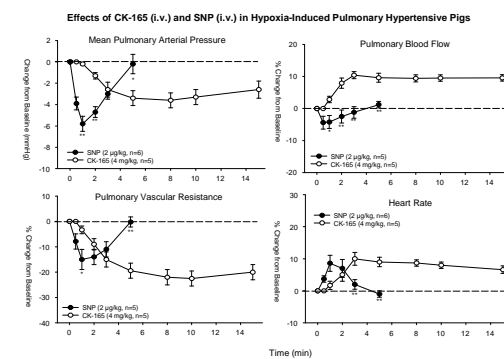


Fig 2: Effects of CK-165 on pulmonary vascular dynamics compared to SNP, in pigs with pulmonary hypertension. Both CK-165 and SNP induced a similar pattern in pulmonary arterial pressure, pulmonary vascular resistance and heart rate. However, the duration of action was more prolonged with CK-165 and actually increased the pulmonary blood flow. (*p<0.05, **p<0.01)

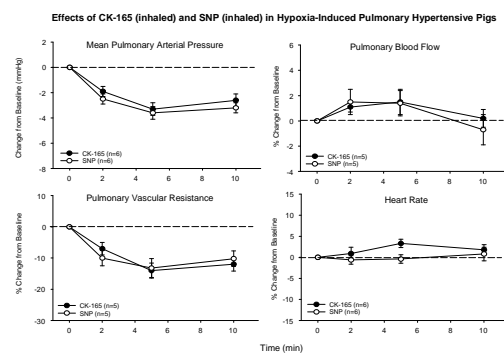


Fig 3: Effects of CK-165 and SNP via inhaled administration on pulmonary vascular dynamics in pigs with pulmonary hypertension. Both CK-165 and SNP induced a similar decreases in pulmonary arterial pressure and pulmonary vascular resistance, and similarly increased pulmonary blood flow. Heart rate was unchanged in both groups.

Table 1
Effects of CK-165 on Pulmonary Hemodynamics in Hypoxia-Induced PHT Pigs

Parameter	Baseline	% Change from Baseline		
		5 min	8 min	15 min
Pulmonary Arterial Blood Flow (L/min)				
CK-165 (2 mg/kg, n=5)	2.8 ± 0.2	+4.4 ± 2.0	+4.1 ± 2.6	+1.4 ± 3.1
CK-165 (4 mg/kg, n=5)	2.8 ± 0.2	+9.6 ± 1.4*	+9.4 ± 1.1*	+9.7 ± 0.9*
CK-165 (6 mg/kg, n=5)	3.0 ± 0.2	+7.4 ± 2.0*	+7.0 ± 2.0	+7.2 ± 1.9
Pulmonary Vascular Resistance (mmHg/L/min)				
CK-165 (2 mg/kg, n=5)	8.0 ± 1.1	-9.9 ± 2.8*	-12.5 ± 2.6*	-8.0 ± 2.1*
CK-165 (4 mg/kg, n=5)	7.7 ± 1.1	-19.4 ± 3.4*	-21.9 ± 2.9*	-19.7 ± 3.0*
CK-165 (6 mg/kg, n=5)	6.8 ± 1.1	-16.7 ± 0.6*	-21.0 ± 3.1*	-20.0 ± 3.0*
Heart Rate (beats/min)				
CK-165 (2 mg/kg, n=5)	146 ± 10	+5 ± 3	+4 ± 3	+2 ± 3
CK-165 (4 mg/kg, n=5)	146 ± 11	+9 ± 1*	+9 ± 1*	+7 ± 1*
CK-165 (6 mg/kg, n=6)	147 ± 12	+10 ± 2*	+8 ± 1*	+7 ± 2

PHT: Pulmonary hypertension; * p<0.05 vs. baseline

2nd Hypothesis

A smooth muscle myosin inhibitor will ameliorate thromboxane induced pulmonary hypertension

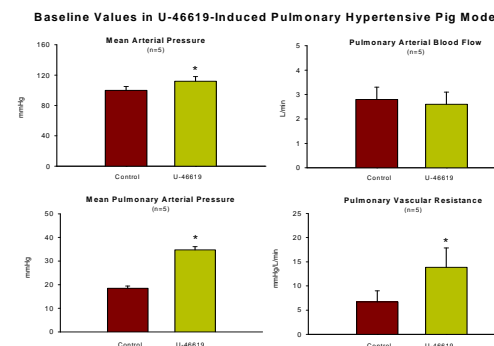


Fig 4: Baseline values of mean arterial pressure, mean pulmonary arterial pressure, pulmonary blood flow and pulmonary vascular resistance in pigs after infusion of U-46619 compared to control. Mean arterial pressure, pulmonary arterial pressure and pulmonary vascular resistance were significantly, p<0.05, increased, in hypertensive condition compared to control.

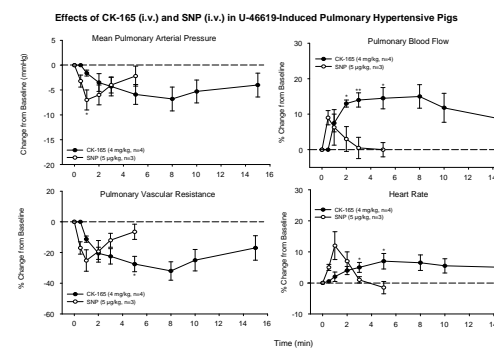


Fig 5: Effects of CK-165 on pulmonary vascular dynamics compared to SNP, in pigs with U-46619-induced pulmonary hypertension. Both CK-165 and SNP induced a similar pattern in all indices measured. However, the duration of action produced by SNP was markedly less compared to CK-165. (*p<0.05, **p<0.01)

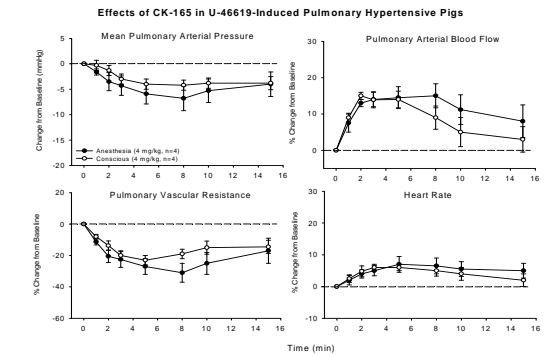


Fig 6: Effects of CK-165 (i.v.) at a dose of 4 mg/kg on pulmonary vascular dynamics in U-46619-induced pulmonary hypertensive pigs in conscious state, as compared to the anesthetized state. Note that the effects of i.v. CK-165 was similar in both states.

Table 2
Effects of CK-165 in Conscious and Anesthetized Pigs with PHT Induced by U-46619

Parameter	Baseline	CK-165 (4 mg/kg, i.v.)		
		5 min	8 min	15 min
Mean Arterial Pressure (mmHg)				
Conscious (n=4)	125 ± 4	-13 ± 2*	-11 ± 1*	-7 ± 1*
Anesthetized (n=4)	98 ± 8	-25 ± 4*	-25 ± 4*	-21 ± 5*
Mean Pulmonary Arterial Pressure (mmHg)				
Conscious (n=4)	31.0 ± 2.6	-3.8 ± 1.0*	-3.9 ± 1.1*	-3.8 ± 1.3
Anesthetized (n=4)	34.1 ± 2.8	-5.2 ± 1.4*	-6.5 ± 2.3	-4.0 ± 2.8
Pulmonary Arterial Blood Flow (L/min)				
Conscious (n=4)	3.1 ± 0.5	+14.0 ± 2.4*	+8.1 ± 3.4	+2.8 ± 3.7
Anesthetized (n=4)	2.2 ± 0.1	+14.5 ± 3.0*	+15.2 ± 3.8*	+8.3 ± 5.7
Pulmonary Vascular Resistance (mmHg/L/min)				
Conscious (n=4)	9.2 ± 1.9	-22.0 ± 3.1*	-18.0 ± 2.9*	-14.7 ± 4.3
Anesthetized (n=4)	14.8 ± 2.1	-25.7 ± 3.9*	-30.7 ± 7.3*	-17.2 ± 9.3
Heart Rate (beat/min)				
Conscious (n=4)	122 ± 10	+6 ± 1*	+5 ± 2	+2 ± 2
Anesthetized (n=4)	139 ± 15	+7 ± 3	+7 ± 3	+5 ± 3

PHT: Pulmonary Hypertension; * p<0.05 vs. baseline
Mean arterial pressure and mean pulmonary arterial pressure: Change from Baseline;
Pulmonary arterial blood flow, pulmonary vascular resistance and heart rate: % Change from Baseline

SUMMARY

- CK-165 delivered systemically at a dose of 4 mg/kg i.v. reduced (p<0.01) pulmonary vascular resistance by 22±3 and 32±6% from their baseline levels in the hypoxia and thromboxane (U-46619) models, respectively. Mean arterial pressure fell modestly and heart rate rose slightly in the conscious state.
- For CK-165 delivered via inhalation, in the hypoxia model, pulmonary vascular resistance fell by a similar degree, 23±3%, while mean arterial pressure and heart rate were unchanged.
- The duration of vasodilatation was relatively longer with CK-165 than with SNP.

CONCLUSION

Inhibition of smooth muscle myosin may be a novel therapeutic approach for the treatment of pulmonary hypertension.