Inhibition of Smooth Muscle Myosin as a Novel Therapeutic Target for Hypertension

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Abbreviations: SMM, smooth muscle myosin; CCB, calcium channel blocker; MAP, mean arterial pressure; CO, cardiac output; LVP, left ventricular pressure; mRA, mean right atrial pressure; RF, renal flow; IF, iliac flow; TPR, total peripheral resistance; RR, renal resistance; IR, iliac resistance; LV, left ventricle; SHR, spontaneously hypertensive rat; AII, Angiotensin II.
ABSTRACT

We examined a novel therapeutic approach for hypertension, a small molecule direct inhibitor of smooth muscle myosin, CK-2018448, in conscious dogs with renal hypertension and compared its efficacy with that of a calcium channel blocker, amlodipine. Dogs were instrumented with a miniature left ventricular pressure gauge, an aortic pressure catheter, and ultrasonic flow probes in the ascending aorta, renal and iliac arteries for measurement of cardiac output and regional blood flow. In the hypertensive state, mean arterial pressure increased from 101±3.8 to 142±1.9 mmHg. At the doses selected, CK-2018448 (CK-448) and amlodipine increased cardiac output similarly (30±11% vs. 33±6.4%) and similarly reduced mean arterial pressure (-22±3.6% vs. -16±3.4%) and total peripheral resistance (-36±5.9% vs. -37±5.8%) similarly. CK-448 had the greatest vasodilator effect in the renal bed, where renal blood flow increased by 46±9.0%, vs. 11±3.4% for amlodipine (p<0.01). CK-488 produced significantly less vasodilation in the limb, where iliac blood flow did not change; in contrast, it rose by 48±12% with amlodipine (p<0.01). The minimal effects on limb blood flow could limit the development of peripheral edema, an adverse side effect of Ca\textsuperscript{2+} channel blockers. Additionally, in a rodent model of hypertension, oral administration of a smooth muscle myosin inhibitor resulted in a sustained antihypertensive effect. Thus, the smooth muscle myosin inhibitor’s preferential effect on renal blood flow makes this drug mechanism particularly appealing, since many patients with hypertension have renal insufficiency, and patients with heart failure could benefit from afterload reduction coupled with enhanced renal blood flow.
INTRODUCTION

Despite the number of available anti-hypertensive agents that are often used in combination coupled with lifestyle modification, blood pressure remains uncontrolled in 56% of patients treated for hypertension (Lloyd-Jones et al., 2010). Accordingly, there is still a need for novel approaches to the treatment of hypertension. Recently, drugs have been developed that directly target myosin. A direct activator of cardiac myosin has demonstrated promise for the treatment of heart failure (Shen et al., 2010; Malik et al., 2011) and is currently in clinical trials (Cytokinetics, 2007-2009; Greenberg et al., 2009; Habibzadeh et al., 2010). Smooth muscle myosin is a mechanochemical enzyme that hydrolyzes ATP to generate mechanical force and is responsible for generating vascular tone (Morano et al., 2000; Babu et al., 2001). Inhibitors of myosin function have been described previously (Watanabe et al., 2010). A direct inhibitor of smooth muscle myosin (SMM) would be attractive for reducing arterial pressure through peripheral vasodilation. A selective, direct smooth muscle myosin inhibitor capable of blocking ATP hydrolysis that relaxes vascular smooth muscle (CK-2018448 (CK-448)) has been developed (Qian et al., 2009; Clancy et al., 2010) and its effect on vascular resistance in a conscious model of hypertension is now described for the first time.

The goal of the current investigation was to assess the efficacy of this novel therapeutic approach by first examining its effects in conscious dogs, chronically instrumented for instantaneous and continuous measurement of cardiac output, arterial pressure, and blood flow in the renal and iliac beds in the setting of renal hypertension (Vatner et al., 1985). A secondary goal was to compare its action with that of a standard antihypertensive drug and known potent vasodilator, which blocks calcium channels, i.e., amlodipine. We were interested in examining the regional vascular effects; the renal bed, because many patients with hypertension have renal
insufficiency; and the iliac bed, because peripheral edema, an undesirable side effect of many
drugs, is intensified by limb vasodilation, especially with calcium channel blockers (CCB)
(Gustafsson, 1987; Gustafsson et al., 1989; Pedrinelli et al., 2000; Messerli, 2002b). The
chronically instrumented canine hypertension model permitted monitoring of direct and
continuous measurements of regional blood flows and cardiac dynamics, in the absence of
anesthesia, which would affect these measurements. The antihypertensive effects of direct SMM
inhibitors were examined further using a hypertensive rat model, i.e. spontaneous hypertensive
rats (SHR). This rodent model was particularly useful for examining the effect of another SMM
inhibitor, CK-2018509 (CK-509), when given orally on sustained blood pressure lowering.
METHODS

Animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals of the Institute (NIH, 2010) and New Jersey Medical School Institutional Animal Care and Use Committee. 15 female mongrel dogs (15-20 kg) were anesthetized with thiopental (15 mg/kg iv) followed by endotracheal intubation and halothane (1.0–1.5 vol%) anesthesia. A laparotomy was performed, the left kidney was exposed and the left renal artery lumen was reduced to a final arterial diameter of 0.9 mm by constricting against a 20 gauge catheter. The right renal artery and left proximal iliac artery were exposed for placement of a Transonic flow probe (Transonic systems Inc.). Through a left thoracotomy Tygon catheters were placed in the descending aorta, and the right atrial appendage to measure their respective pressures. A solid-state miniature pressure transducer (Konigsberg) was implanted in the left ventricle (LV) via the apex for LV pressure measurement. Transonic flow probes were placed around the root of ascending aorta to measure cardiac output. The chest and abdomen were closed and post-operative analgesics were administered until the animals recovered fully from surgery.

Spontaneously hypertensive rats (SHR, age approximately 12 wks) were purchased from Charles River Laboratory (Portage, MI). For intravenous dosing experiments, rats were anesthetized with isoflurane and femoral artery and vein cannulated. Drug was administered as a slow bolus over 2 minutes via the femoral vein. For oral dosing experiments, SHR rats were anesthetized and cannula implanted in right jugular vein for drug delivery, and the right carotid artery for arterial pressure monitoring. Animals were allowed to recover and underwent restrainer acclimatization prior to oral dosing and blood pressure measurement. For IV dosing, CK-2018509 was formulated as a solution in 50% polyethylene glycol 400 and 50%
hydroxypropyl beta cyclodextrin. For oral dosing, CK-2018509 was formulated in 10% dimethylacetamide and 90% polyethylene glycol 400 and given by oral gavage. All procedures and protocols were approved by the Cytokinetics, Inc. Institutional Animal Care and Use Committee and followed the National Institutes of Health guidelines for the care and use of laboratory animals.

**Hemodynamic measurements.** Systemic hemodynamics in dogs were recorded using a signal amplifier (Triton, system 6, model 200) and PowerLab data acquisition system (ADInstruments, Inc.). Aortic and right atrial pressures were measured using strain gauge manometers that had been calibrated with a mercury manometer connected to the fluid-filled catheters. The solid-state left ventricular (LV) pressure gauge was cross-calibrated with aortic pressure measurements. Cardiac output (CO), renal flow (RF) and iliac flow (IF) were measured with a Transonic flow meter (Transonic system Inc., T206). Total peripheral resistance (TPR), renal resistance (RR) and iliac resistance (IR) were calculated: (mean arterial pressure - right atrial pressure) / corresponding blood flow. Some flow probes did not work properly, and accordingly data could not be collected in some cases for the measurements of cardiac output (n=2), renal flow (n=2), and iliac flow (n=2).

In SHR, blood pressure during intravenous dosing was measured with a solid-state pressure transducer (SPR-320, Millar Instruments, Houston, TX) advanced retrograde via the femoral artery into the descending aorta using a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO). During oral dosing, rats were placed in restrainers for blood pressure monitoring; blood pressure was measured via the carotid artery cannula with a fluid-filled dome pressure transducer (MLT844, ADInstruments) using a PowerLab data acquisition system.
**Experimental protocol for CK-448.** Before and during the post-operative recovery period, the dogs were trained to lie quietly in the right lateral position. Hemodynamic parameters and arterial pressures were monitored every other day for 2 weeks until mean arterial pressure was consistently elevated to a level higher than 130 mmHg for at least 3 days, indicating the development of stable hypertension. In 4 dogs, the arterial pressure did not begin to increase immediately after surgery and the baseline data from those 4 dogs were used for the normotensive baseline. In order to achieve a significant elevation of arterial pressure over baseline (before hypertension), continuous angiotensin II (AII) infusion at a dose of 10-15 ng/kg/min was applied in some of the dogs. Prior to the initiation of the acute experiment, an intravenous catheter (19 gauge) was implanted in the saphenous vein of the hind leg for drug administration. An optimal dose of CK-448, 4mg/kg i.v. bolus, was selected based upon stable dose effects observed during the preliminary dose response study (Figure 1). To determine whether autonomic reflexes affected the hemodynamic properties, CK-448 was also administered to 5 normotensive dogs along with autonomic blockade using hexamethonium (30mg/kg iv), propranolol (1mg/kg iv) and atropine (0.1mg/kg iv) bolus. The effects of CK-448 were also compared with those of a known vasodilator used in hypertension therapy, i.e., amlodipine. The dose of amlodipine (Pfizer Inc.), 0.2mg/kg i.v. was selected to match the CK-448 dose. Drug responses for CK-448 and amlodipine were continuously monitored for 30min. Peak responses were analyzed using the averaged data from the first 8 min after drug administration. Vehicle (saline, pH 7.4) was administered to 3 hypertensive dogs matching the volume of CK-448 administered. CK-448 is an N, N'-alkylurea (US Patent Application No. 20098/0275537) and was provided by Cytokinetiics, Inc.
**Experimental protocol for CK-509**: SHRs were administered CK-509 10mg/kg i.v. and arterial pressures were monitored continuously for 60min. For oral administration of CK-509, SHRs received a dose of 200 mg/kg on the first day (t=0) and then 50 mg/kg at 24 and 36 hours; arterial pressures were monitored for 48 hours at discrete timepoints. In control groups, vehicle (50% polyethylene glycol 400 and 50% Hydroxypropyl beta cyclodextrin) for intravenous dosing and (10% dimethylacetamide and 90% polyethylene glycol 400) oral dosing were administered to SHRs via the same methods and in matching volumes as that of CK-509.

**Statistical analysis**. Data are expressed as mean ±SE. Statistical significance was determined using two sample t test. Wilcoxon rank sum test was employed to determine significance in renal flow due to big variation in amlodipine group. In the dog experiments, peak response time was chosen based on continuous monitoring and accordingly, data from first 8 min after drug administration were averaged.
RESULTS

Systemic Hemodynamics in conscious dogs before and after hypertension.

In the hypertensive state, mean arterial pressure (MAP) increased from 101±3.8 to 142±1.9 mmHg (p<0.01, data not shown), while cardiac output fell by 50±4.9% and total peripheral resistance (TPR) rose by 206±34%. LV systolic pressure increased by 37±3.2%, heart rate did not change significantly (96±6.1 to 106±6.9 beats/min), and mean right atrial pressure remained similar between the two groups (4.7±1.4 vs. 3.4±0.7 mmHg, data not shown). Vascular resistances rose in the renal and iliac beds (data not shown).

Anti-hypertensive effect of CK-448 vs. amlodipine. Doses of CK-448 (4 mg/kg i.v. bolus) and amlodipine (0.2 mg/kg i.v. bolus) were selected to decrease MAP and TPR similarly; at these doses heart rate and cardiac output increased similarly with the two drugs at peak response (Table 1, Figure 2). Peak responses were determined using the averaged values from first 8 min after drug administration. Administration of vehicle did not have an effect on TPR (data not shown).

Effects on regional beds after CK-448 compared to amlodipine. CK-448 increased renal flow (46±9.0%), but did not increase iliac flow (Table 2, Figure 2). Amlodipine exerted a markedly different effect on the regional beds, where the greatest increase occurred in iliac blood flow (48±12%), but renal blood flow did not increase much (Table 2, Figure 2).

CK-448 decreased renal resistance significantly more than amlodipine (-46±4.8% vs. -25±5.4%, p<0.05), while amlodipine decreased iliac resistance more profoundly than CK-448 (-42±5.5% vs. -23±4.6%, p<0.05) (Table 2). Administration of vehicle did not have an effect on the regional beds (data not shown).
The effects of CK-448 were compared in the hypertensive dogs with and without supplemental AII: CK448 increased RBF similarly with (14ml/min) or without (16ml/min) supplemental AII, but did not increase IBF in either group.

**Effects of CK-448 after autonomic blockade in normotensive dogs.** After autonomic blockade, CK-448 no longer increased heart rate and LV dP/dt but reduced mean arterial pressure to a greater extent (-33±1.3% vs. -11±1.4%). While, autonomic blockade did not affect the decrease in renal vascular resistance (-43±2.9% vs. -39±1.8%, figure 3), it did produce greater vasodilation in the iliac bed (-30±4.8%) as compared to the results in the absence of autonomic blockade (-12±1.2%, Figure 3).

**Antihypertensive effects of CK-509 in hypertensive rats.** Unlike CK-448, the SMM inhibitor, CK-509 has the advantage of oral administration. Using SHRs, first we demonstrated that direct i.v. administration of CK-509 (10mg/kg i.v. bolus) reduced MAP (Figure 4A). Repeat oral administration of CK-509 produced a sustained reduction in mean arterial pressure over 2 day period (Figure 4B).
DISCUSSION

Despite life style modification, the large number of available pharmacologic agents, and combination therapies, the blood pressure of many hypertensive patients is not well controlled, suggesting the need for novel therapeutic mechanisms. The current investigation of the selective, direct smooth muscle myosin inhibitor, CK-448, was conducted in the chronically instrumented conscious dog model of renal hypertension, with continuous measurements of cardiac output, blood flows and pressures (Knight et al., 1985; Kirby and Vatner, 1987; Gelpi et al., 1988; Gelpi et al., 1991). This model gave a comprehensive initial picture of the hemodynamic profile of this novel anti-hypertensive mechanism.

As expected, the SMM inhibitor reduced arterial pressure in both hypertensive dogs and rats. Using the instrumented large animal model of renal hypertension, we were able to observe not only the decrease in mean arterial pressure but also to measure a decrease in total peripheral resistance, and increase in cardiac output. However, the hemodynamic profile was not as predicted based on prior experience. Most vasodilators either affect all peripheral beds similarly (Levine et al., 1984; De Angelis et al., 2005) or elicit preferential vasodilation in the limbs and skeletal muscle (Gavras and Liang, 1980; Mancia and Ferrari, 1992; Shen and Vatner, 1993). The SMM vasodilator we studied exerted an unusual, but potentially desirable, effect on blood flow distribution and regional vasodilation. In particular, this new drug increased renal blood flow markedly by inducing renal vasodilation. Although some of the dogs required supplemental AII infusion to produce hypertension, subgroup analysis showed similarly improved renal blood flow without significant change in iliac blood flow in renal hypertension animals with or without AII infusion. Furthermore, the renal vasodilation was not affected by autonomic blockade, indicating that the renal vasodilation was a direct effect on the renal vascular bed and not
buffered by autonomic reflexes. In contrast, vasodilation in the limb was greater after autonomic blockade, due to alleviation of the reflex constriction in the limb induced by the fall in arterial pressure and unloading of the arterial baroreflex resulting in increased peripheral alpha adrenergic vasoconstriction in the limb. The fact that the renal vasodilation was not enhanced after autonomic blockade could be due to several factors. First, reflex increases in vasoconstrictor tone are generally thought to be less in the kidney than in the limb. It is also possible that the SMM inhibitor blocked renal autoregulation, a powerful mechanism important for maintaining blood flow to the kidney in response to alterations in arterial perfusion by modulating renal vascular smooth muscle tone. Equally important, there was no significant increase in iliac blood flow supplying muscle, bone and skin, which is favorable, since this adverse effect could intensify peripheral edema (Salmasi et al., 1991; Messerli, 2002a).

The second goal of the current study was to compare the effect of the novel SMM vasodilator, with another potent vasodilator, that is commonly used in the treatment of hypertension, a calcium channel blocker, amlodipine. When the two drugs were compared in the hypertensive dogs at doses that increased cardiac output and reduced arterial pressure and total peripheral resistance equally, there was a marked difference in blood flow distribution and regional vascular resistance. The calcium channel blocker had the opposite effect from the SMM inhibitor. The calcium channel blocker did not increase renal blood flow, but increased iliac blood flow markedly. As mentioned above, this could have the adverse consequences of intensifying peripheral edema, since it is known that this can occur with an increase in limb blood flow (Rendell et al., 1997; Leonetti et al., 2002; Messerli, 2002a) and is frequently observed with chronic administration of calcium channel blockers (Rendell et al., 1997; Leonetti et al., 2002). There were no major differences in systemic effects.
Based upon the results of the current study, smooth muscle myosin inhibition could be a promising novel strategy for the treatment of hypertension, because these patients should benefit from an increase in renal blood flow. Furthermore, since it did not increase iliac blood flow, it is likely that peripheral edema, an adverse side effect of calcium channel blockers, would not occur with smooth muscle myosin inhibition.

One limitation of the SMM inhibitor used in the canine study is that it is only currently available for i.v. administration, thereby limiting its applicability to the general hypertensive population. However, even in its current i.v. formulation, this novel agent may be suited for use in hypertensive crises or in malignant hypertension, when rapid and controlled lowering of arterial pressure is required.

Oral administration is required for therapy in the majority of hypertension patients. Accordingly, we also examined the effects of a different SMM inhibitor, CK-509, which was suitable for oral administration in animal models. The initial studies in SHR showed that CK-509 administered orally could produce an antihypertensive effect lasting for an entire 2 day observation period.

In conclusion, the preferential increase in renal blood flow with the SMM inhibitor is particularly appealing for hypertension therapy, since many of the patients have renal insufficiency. Future studies will need to establish the potential benefit of SMM inhibition on end-organ damage in hypertension. Although we did not directly examine the effects of SMM inhibition on renal function, an increase in renal perfusion should be salutary not only in patients with hypertension, but also, in patients with heart failure, and particularly those with cardio-renal syndrome, who could benefit from the combination of afterload reduction and increased renal perfusion.
Acknowledgements:

None

Authorship Contribution:

Participated in research design: Zhao, Malik, Morgans, Vatner

Conducted experiments: Zhao, Vatner, Wang, Jia, Pannirselvam,

Contributed new agents: Morgans, Malik

Performed data analysis: Zhao, Ho, Malik, Dhar, Wang, Jia, Pannirselvam,

Wrote the manuscript: Zhao, Ho, Malik, Vatner, Abarzua
REFERENCES


Cytokinetics (2007-2009) A Phase II, Multi Center, Double-Blind, Randomized, Placebo Controlled, Dose-Escalation, Pharmacokinetic (PK) and Pharmacodynamic (PD) Study of CK-1827452 in Patients With Stable Heart Failure, in, ClinicalTrials.gov.


FOOTNOTE

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DISCLOSURES:

The authors have nothing to disclose except for Drs. Malik, Morgans, Wang, Jia, and Pannirselvam who are employees or former employees of Cytokinetics, Inc., and Dr. Abarzua is an employee of CV Dynamics, Inc. Dr. Vatner has stock in CV Dynamics, Inc.
FIGURE LEGEND

Figure 1  Dose response of CK-448 in conscious normal dogs. Cardiac output, renal flow and iliac flow were compared with CK-448 2, 4, 6mg/kg i.v. bolus. (n=5)

Figure 2  Time course comparing the effects of Amlodipine and CK-448 on cardiac output, renal and iliac artery flows in conscious hypertensive dogs. Percent changes from baseline (B) are compared between CK-448 and Amlodipine for 30min (n=5 in Amlodpine, n=7 in CK-448) *p<0.05, **p<0.01). CK-448 increased renal blood flow, but not the iliac blood flow. In contrast, amlodipine increased iliac flow significantly.

Figure 3  Percent changes from baseline at 3 minutes after CK-448 with and without autonomic blockade in conscious normotensive dogs. Mean arterial pressure (AR), heart rate (HR), total peripheral resistance (TPR), (LV dP/dt, renal resistance (RR) and iliac resistance (IR) were compared in the presence and absence of autonomic blockade. Autonomic blockade eliminated the reflex increases in heart rate and LV dP/dt and induced a greater fell in arterial pressure, but did not affect the reduction in renal vascular resistance. (n=4 in “with blockade” group, n=5 in “without blockade” group). **p<0.01 with blockade different from without blockade.

Figure 4  A: Effects of CK-509 (10mg/kg i.v. bolus) in SHR, showing reduction in mean arterial pressure (MAP) (n=5). *p<0.05 vs. vehicle from 1 minute after drug administration through 1 hour. B: Oral administration of CK-509 (200mg/kg on Day 1 followed by 50 mg/kg twice daily) in SHR (n=4) also reduced MAP over a 2 day monitoring period. *p<0.05 vs. vehicle (n=8) beginning at 1 hour of treatment through 48 hours.
Table 1. Hemodynamic Measurements after Hypertension and during Peak Drug Response

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<td>CK 448</td>
<td>112±6.3</td>
<td>34±4.8**</td>
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<td><strong>Arterial Systolic Pressure (mmHg)</strong></td>
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<td>-37±5.8**</td>
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*p<0.05, **p<0.01 from hypertension baseline

#Percent change from baseline comprised of the average of the first 8 minutes after drug administration
Table 2. Blood Flow Effects after Hypertension and during Peak Drug Response

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<td>CK 448</td>
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*p<0.05, **p<0.01 from hypertension baseline

††p<0.01 compared to Amlodipine

# Percent change from baseline comprised of the average of the first 8 minutes after drug administration
Figure 1

Dose Response of CK-448 in Conscious Dogs

Cardiac Output

Renal Flow

Iliac Flow
Figure 2

Blood Flow Effects Comparison of CK-448 and Amlodipine

Cardiac Output

Renal Flow

Iliac Flow

% Change

Time (min)
Figure 3: Effects of Autonomic Blockade on CK-448 Responses

- MAP
- HR
- LV dP/dt
- TPR
- RR
- IR

Comparison between conditions with and without blockade, showing significant changes.
Figure 4

I.V. and Oral Effect of CK-509

A. I.V. administration of CK-509 on SHR

B. Oral CK-509 - SHR

MAP (mmHg)

Time (hours)

Vehicle
CK-509

*