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Subdose of fasudil suppresses myocardial fibrosis in aldosterone-salt-treated uninephrectomized rats

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Rho/Rho kinase (ROCK) pathway plays an important role in pathological cardiovascular conditions. In the present study, the effect of a subdose of fasudil, a selective ROCK inhibitor, on systemic hypertension and myocardium fibrosis induced by aldosterone was investigated in uninephrectomized Sprague-Dawley rats (SD). Treatment with a fasudil (10 mg/kg·day, s.c.) for 5 weeks decreased the activity of ROCK activity for more than 53% as determined by the expression of phosphorylated Myosin phosphatase target subunit 1 (MYPT1). Although this dose of fasudil did not signifantly prevent hypertension, it remarkably alleviated myocardium hypertrophy and fibrosis. The elevated transcriptional expression of transforming growth factors β1 (TGF-β1), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and collagen I and III was also decreased. These results demonstrated that fasudil can protect the myocardium from injury by aldosterone at a subhypertensive dose.

1. Introduction

Chronic presure overload due to hypertension leads to myocardial hypertrophy and heart failure (Guo et al. 2006). Recently, Rho/Rho kinase (ROCK), a serine/theronine kinase which is activated by small GTPase RhoA, has attracted much attention in the cardiovascular research field due to its important roles in various cellular functions that are involved in the pathogenesis of cardiovascular diseases (Dong et al. 2010; Lai and Frishman 2005; Uehata et al. 1997). ROCK phosphorylates the myosin phosphatase target subunit (MYPT) of myosin regulatory light chain phosphotase (MLCP), a key molecular in the activation of actomyosin and the regulation of smooth muscle contraction (Shichi et al. 2010). It also plays important roles in the development of cardiac remodeling, left ventricular (LV) hypertrophy and myocardial fibrosis (Funder 2010; Haider et al. 2003). Therefore, ROCK has emerged as a promising drug target for various cardiovascular diseases that are frequently associated with hypertension and cardiac remodeling (Connolly and Aaronson 2011; Fukui et al. 2008; Shimokawa and Takeshita 2005).
In a series of experimental studies, fasudil, the selective Rho kinase inhibitor, has been shown to exhibit many beneficial effects on the cardiovascular system under pathological conditions. It markedly attenuates hypoxic pulmonary vasoconstriction and ameliorates the the development of pulmonary hypertension (Dai et al. 2011; Fagan et al. 2004; Fujita et al. 2010; Mouchaers et al. 2010). It also alleviates myocardial fibrosis (Ishimaru et al. 2007) and angina (Shimokawa et al. 2002), and prevents angiotensin II- induced cardiac hypertrophy (Wang et al. 2005). Although the anti-pulmonary hypertension activity of fasudil has been intensively investigated (Chen et al. 2009; Dai et al. 2011; Fujita et al. 2010; Li et al. 2007, 2011; Mouchaers et al. 2010; Tourneux et al. 2008), investigations on the effects of fasudil on systemic hypertension are relative scarce.
In the present study, the effects of a subdose of fasudil on systemic hypertension was investigated in aldosterone-salt-treated uninephrectomized SD rats. The beneficial effects of fasudil on myocardial hypertrophy and fibrosis under chronic hypertension were particularly addressed.

2. Investigations and results

2.1. Subdose of fasudil does not suppress hypertension induced by aldosterone

As determined by the expression of phosphorylated MYPT1, the major substate of ROCK in smooth muscle (Shichi et al. 2010), treatment with aldosterone greatly activated ROCK and subquently increased the systolic blood pressure (SBP) of uninephrectomized rat (Figs. 1 and 2). After treatment with fasudil (10 mg/kg·day, s.c.) for 5 weeks, the activation of ROCK by aldosterone was substantially suppressed (Fig. 1), but the enhancement of SBP was not significantly alleviated (Fig. 2). Therefore, fasudil cannot supress systemic hypertension induced by aldosterone in a relative low dose.

2.2. Subdose of fasudil remarkably alleviates left ventricular (LV) hypertrophy and myocardial fibrosis

Table 1 shows the effects of aldosterone and fasudil on body weight and cardiac function in uninephrectomized rats. Administration of aldosterone significantly increased LV

Table 1: Effects of aldosterone and fasudil on body weight (BW), left ventricular (LV) weight and cardiac function and hemodynamics in uninephrectomized rats treated with 1% NaCl for 5 weeks

	Vehicle (2% alcohol) n = 8	Aldosterone (0.75 μg/H) n = 9	Aldosterone + fasudil (10 mg/kg/day) n = 8
BW (g)	513 ± 10	451 ± 9*	460 ± 12*
LV weight (mg)	925 ± 50	1350 ± 80*	1100 ± 40*†
LV/body weight (mg/g)	1.8 ± 0.2	3.0 ± 0.3*	2.4 ± 0.1*†
Fractional shortening (%)	47 ± 5	74 ± 6*	60 ± 3*†
LVEDd (mm)	6.9 ± 0.4	6.5 ± 0.5	6.9 ± 0.5
PWd (mm)	1.8 ± 0.2	2.4 ± 0.1*	1.9 ± 0.1†

Values are means ± SE. *P< 0.05 vs. vehicle. † P< 0.05: aldosterone vs. aldosterone + fasudil. LVEDd, LV end-diastolic dimension; PWd, posterior wall thickness at end-diastole.

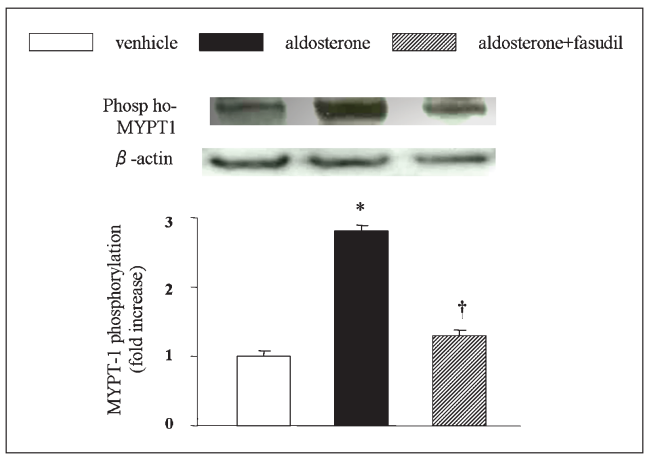

Fig. 1: Subdose of fasudil significantly inhibited the activity of ROCK induced by aldosterone. The activity of ROCK was determined by the protein level of phosphorylated MYPT1, the major substate of ROCK in smooth muscle. The histogram below presented the quantative data for the western blot results that was calculated by ImageJ 4.0.

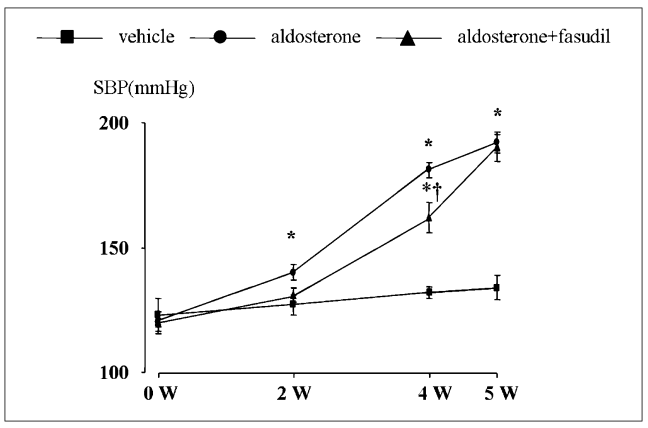

Fig. 2: The time-course effects of subdose of fasudil on the systolic blood pressure (SBP). All animals were uninephrectomized and given high sodium diet (1% NaCl in the drinking water) during the experiment.

weight, LV/body weight, factional shortening and posterior wall thickness at end-diastole, revealing a signficant myocardial hypertrophy-stimulating effect. Treatment with a subdose of fasudil remarkably alleviated the myocardial hypertrophy induced by aldosterone (Table 1). Meanwhile, the myocardial fibrosis induced by aldosterone was also substentially inhibited in rat treated with fasudil (Fig. 3). These data displayed the beneficial effect of subdose of fasudil on cardiac function under chronic hypertension.

2.3. Subdose of fasudil significantly inhibits the transcriptional expression of biomarkers for myocardial fibrosis

We further investigated the effects of fasudil on the expression of ANP, BNP, TGF-β1, collagen I and III, the five important myocardial fibrosis markers (Hermida et al. 2009; Ho et al. 2010; Koitabashi et al. 2007; Pei et al. 2010). As shown in Fig. 4, ANP, BNP, TGF-β1 and collagen I and III mRNA levels were significantly elevated in myocardial tissues of aldosterone-treated rats as compared with the vehicle control. Treatment with a subdose of fasudil markedly attenuated the elevation of these mRNA expression in uninephrectomized rats (Fig. 4), suggesting a general regulation of fasudil on myocardial fibrosis under chronic hypertension.

3. Discussion

In patients with hypertension, chronic pressure overload often leads to LV hypertrophy and myocardial fibrosis (Gradman and Wilson 2009; Simko and Pechanova 2010). In recent years, fasudil, a selective inhibitor of ROCK, has been demonstrated in many studies to exhibit beneficial effects on cardiac function under pathological conditions (Demiryurek et al. 2005;

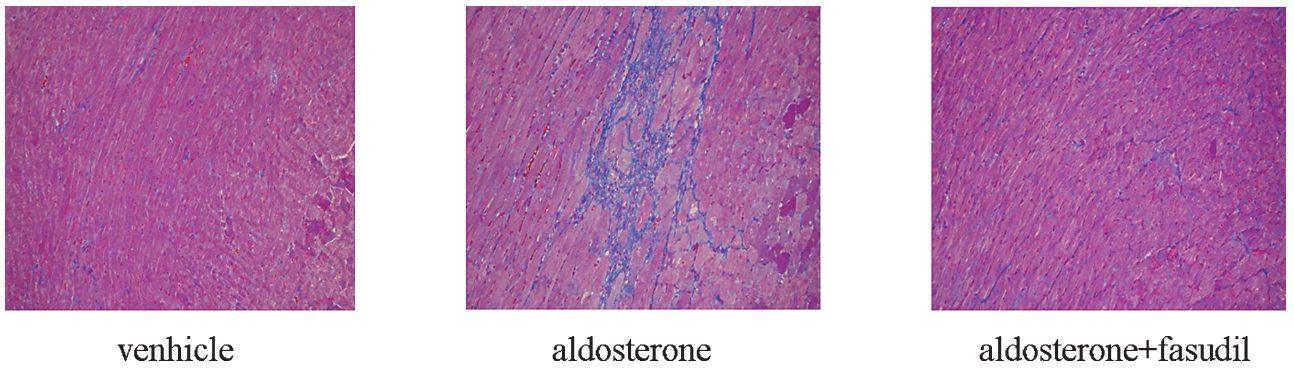

Fig. 3: Subdose of fasudil remarkably alleviated the myocardial fibrosis induced by aldosterone. All animals were uninephrectomized and given high sodium diet (1% NaCl in the drinking water) during the experiment. The myocardium sections (2–3 μm thick) were HE stained and photographied by a OLYMPUS DP70 image analyzer.

Table 2: Oligonucleotide primers for realtime PCR

	Sense	Anti-sense
TGF-β1	CAAAGACATCACACACAGTA	AGGTGTTGAGCCCTTTCCAG
ANP	GGCTCCTTCTCCATCACCAA	TGTTATCTTCGGTACCG
BNP	CTGGGAAGTCCTAGCCAGTCTCCA	GCGACTGACTGCGCCGATCCGGTC
collagen I	CCAGCCGCAAAGAGTCTACATGTC	TCACCTTCTCATCCCTCCTAA
collagen III	TGCCCACAGCCTTCTACACCT	CAGCCATTCCTCCCACTCCAG
GADPH	TCCCTCAAGATTGTCAGCAA	AGATCCACAACGGATACATT

Fukumoto et al. 2007; Otsuka et al. 2008; Wang et al. 2005). In 2005, Wang et al. reported that treatment with fasudil (136–213 mg/kg) dose-dependently attenuated angiotensin II-induced cardiac hypertrophy, prevented perivascular fibrosis, blunted the increase in ANP and collagen type III expression, and improved cardiac function, without changing blood pressure. More recently, another group investigated the effect of a lower dose of fasudil (10 mg/kg) on myocardial fibrosis induced by deoxycorticosterone-acetate (Ishimaru et al. 2007). They found that treatment with fasudil for 28 days did not significantly decrease the systolic blood pressure (SBP), but attenuated the extent of myocardial fibrosis and mRNA levels of procollagen I, procollagen III and plasminogen activator inhibitor-1. The transcriptional expression of TGF-β1 was also decreased by fasudil but with no significance (Ishimaru et al. 2007).

In the present study, the effects of a subdose of fasudil on systemic hypertension and myocardial fibrosis were reconfirmed in uninephrectomized rats treated with aldosterone and high salt diet. In accordance with previous reports, treatment with 10 mg/kg of fasudil for 5 weeks did not significantly prevent the development of systolic hypertension induced by aldosterone (Fig. 2). However, this sub-antihypertensive dose of fasudil markedly alleviated the LV hypertrophy and myocardial fibrosis (Fig. 3 and Table 1). We further determined the mRNA levels several myocardial fibrotic biomarkers, TGF-β1, ANP, BNP, collagen I and III, in tissue samples from different groups. As expected, the increase of transcriptional expression of ANP, BNP, collagen I and III, induced by aldosterone were all largely decreased (Fig. 4). The mRNA level of TGF-β1 was also significantly decreased by the treatment with fasudil (Fig. 4) which was different to the report by Ishimaru et al. (2007). This was likely due to the different animal model and the longer treatment in our study.

Overall, the results we presented here demonstrated that fasudil can ameliorate LV hypertrophy and myocardial fibrosis induced by aldosterone even in a sub-antihypertensive dose.

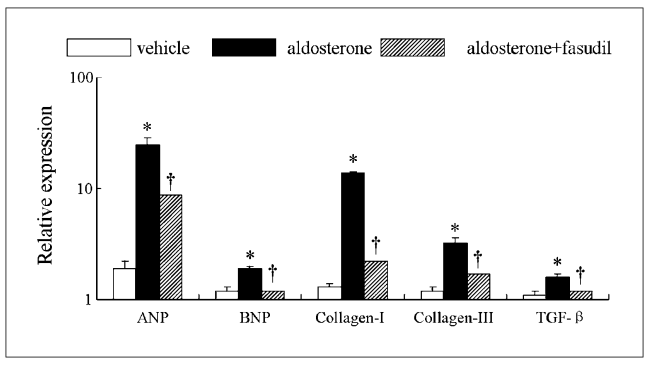

Fig. 4: The effects of subdose of fasudil on mRNA levels of heart TGF-β1, ANP, BNP, collagen I and III. All animals were uninephrectomized and given high sodium diet (1% NaCl in the drinking water) during the experiment. The mRNA levels of different genes were determined by realtime PCR with GADPH as internal standard.

4. Experimental

All experiments were performed according to the guidelines of the Chinese Academy of Medical Science for Institutional Animal Care. Twenty-six healthy male SD rats, weighing 180–200 g, were uninephrectomized at 5 weeks age and divided into 3 groups randomly. All rats were given high sodium diet (1% NaCl in the drinking water) during the experimental period. The aldosterone group received a subcutaneous aldosterone administration (0.75 μg/h, dissolved in 2% alcohol). The control group was given equal volume of 2% alcohol. And the fasudil group was given aldosterone (0.75 μg/h, dissolved in 2% alcohol) and fasudil (10 mg/kg·day, s.c.). Aldosterone and alcohol were subcutaneously administrated via osmotic minipumps (model 2002; Alza Co, Palo Alto, CA) for 5 weeks.

Body weight was determined once a week. The systolic blood pressure (SBP) was measured at 0, 1, 3 and 5 weeks after fasudil treatment with a noninvasive tail-cuff system (BP-98A, Softron Co,Tokyo, Japan) as previously described (Guo et al. 2006). Plasma and myocardial tissue were collected at the end of the experiment. Histological samples preparation and observation were performed as previously reported (Lebrasseur et al. 2007). The western blot for phospho-MYPT1 and the realtime PCR for TGF-β1, ANP, BNP and collagen I and III, were performed routinely with β-actin and GADPH as internal control for western blot and realtime PCR (Table 2) respectively.

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