Increased Rho kinase activity in mononuclear cells of dialysis and stage 3-4 chronic kidney disease patients with left ventricular hypertrophy: cardiovascular risk implications

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Abstract

Cardiovascular disease (CVD) is the leading cause of excess mortality in chronic kidney disease (CKD) and dialysis patients (DP) who have higher prevalence of left ventricular hypertrophy (LVH), the strongest predictor of CV events. Rho kinase (ROCK) activation is linked in hypertensive patients to cardiac remodeling while ROCK inhibition suppresses cardiomyocyte hypertrophy and, in a human model opposite to hypertension such as Bartter's and Gitelman's syndromes patients, its downregulation associates with lack of CV remodeling. Information on ROCK activation-LVH link in CKD and DP is lacking.

Mononuclear cells (PBMCs) MYPT-1 phosphorylation, a marker of ROCK activity, and the effect of fasudil (500 and 1000 µM), a ROCK inhibitor, on MYPT-1 phosphorylation were assessed in 23 DP, 13 stage 3-4 CKD and 30 healthy subjects (HS) by Western blot. LV mass was assessed by M-mode echocardiography. DP, aged between 42 and 75 years, 16 males and 7 females, were under chronic dialysis treatment with 210-240 min, three times a week with low-flux bicarbonate-dialysis with ultrapure dialysate, using a polysulphone dialyser 1.8 m^2 bicarbonate-dialysis for at least 1 year (range 1-5 years) and vascular access was through the artero-venous fistula; mean Kt/V ratio was: 1.43 ± 0.07. CKD patients were recruited from our outpatient ambulatory, 8 males and 5 females aged between 45-70 years. HS, age range 31-65 years, 20 males and 10 females, recruited from the staff of our Division of Nephrology, Dialysis and Transplantation were used as control group. DP and CKD had higher MYPT-1 phosphorylation compared to HS ($p<0.001$ and $p=0.003$). Fasudil (500 and 1000 µM) reduced MYPT-1 phosphorylation in DP ($p<0,01$). DP had higher LV mass than CKD ($p<0.001$). MYPT-1 phosphorylation was higher in patients with LVH ($p=0.009$) and correlated with LV mass both in DP and CKD with LVH ($p<0.001$ and $p=0.006$, respectively).

The current study provides evidence linking the activation of ROCK, as reflected by the increased phosphorylation state of MYPT-1, to cardiac hypertrophy in dialysis and stage 3-4 CKD patients, a human clinical population at high risk for cardiovascular morbidity and mortality. The results of this study join those provided in another high risk for cardiovascular disease patients such as hypertensive patients and receive indirect support from data provided by a human model opposite to hypertension and type II diabetic patients identifying ROCK activation as a potential LVH marker and providing further rationale for ROCK activation inhibition as target of therapy in patients at high risk for cardiovascular disease such as CKD, dialysis, hypertensive and diabetic patients.

Background

LVH is very prevalent in ESRD and dialysis patients and represents the single strongest predictor of adverse cardiovascular events. In vivo and in vitro studies indicate association of myocardial oxidative stress with myocardial remodeling [1]. CKD and dialysis patients are exposed to increase oxidative stress particularly reactive oxygen species (ROS), which arise from activation of endothelial cells, reduction of antioxidant systems, and ROS mediated destruction of vasodilatory NO.

RhoA/Rho kinase (ROCK) pathway is known to upregulate NADPH oxidases thereby increasing ROS levels and RhoA/ROCK signaling is deeply involved in the cardiovascular effects of oxidative stress [2]. Recently has been reported that ROCK activity is higher in hypertensive patients with LVH indicating that ROCK activation is likely related to pathologic cardiac remodeling and might have a role as LVH marker [3]. In addition, in a human model opposite to hypertension such as Bartter's and Gitelman's syndromes patients, RhoA/ROCK downregulation associates with lack of CV remodeling [4,5]. Information on ROCK activation-LVH link in CKD and DP is lacking. Inibition of RhoA/ROCK pathway has been suggested to lead to cardiovascular protection by in vitro and in vivo studies in rats with Ang II-induced LVH and cardiomyocyte hypertrophy [6].

Aims and Objectives

Although there is accumulating evidence on the role of ROCK activation in the development of hypertension and cardiovascular remodeling, there is limited information regarding the role of ROCK activation in LVH in CKD and dialysis patients, who are recognized CVD high-risk patients.

Patients

- Twenty three patients, aged between 42 and 75 years, 16 males and 7 females, from the Division of Nephrology, Dialysis and Transplantation, Department of Medicine, University of Padova-Padova University Hospital, ***undergoing chronic dialysis treatment*** with 210–240 min, three times a week with low-flux bicarbonate-dialysis with ultrapure dialysate, using a polysulphone dialyser 1.8 m^2 bicarbonate-dialysis for at least 1 year (range 1–5 years) and 13 ***stage 3–4 CKD*** patients, 8 males and 5 females.
- Thirty healthy subjects age range 31–65 years 20 males and 10 females from the staff of Division of Nephrology, Dialysis and Transplantation and Department of Medicine at the University of Padova and Padova University Hospital were used as control group.

BP range	Antihypertensive treatment	Epoetin treatment	Vit D and Ca supplements	Lipid lowering treatment	Folic Acid (dialysis patients)
138/84 to 152/92	-Dihydropiridine calcium channel blockers -ACE inhibitors -α-blockers	4,000 to 12,000 UI/week for dialysis patients 10,000 UI/week for CKD	-10 patients: vit D -13 patients PO4 binders (Sevelamer HCl, Calcium carbonate, lanthanum carbonate)	NONE	ALL Patients 10mg after dialysis session

Methods

- Peripheral blood mononuclear cells (PBMCs) from 20 ml of EDTA anticoagulated blood isolated by Lympholyte-H.
- Analysis of **myosin phosphatase target protein-1 (MYPT-1) phosphorylation** by western blot.
- **ROCK inhibition with *fasudil***: $5x10^6$ PBMCs from 4 representative dialysis patients were incubated with different concentrations of fasudil, inhibitor of ROCK activity: 0 (vehicle), 500 and 100 µM, for 1 hour at 37°. Total protein extracts were then analyzed by western blot.
- **Echocardiography**: left ventricular mass was measured at M-mode echocardiography within 7 days after enrollment normalizing for body surface area and normal values were defined according to guidelines: <116 g/m^2 for males and <96 g/m^2 for females.
- **Statistical anlysis**: data are expressed as mean ± SD; Gaussian distribution assessed by Kolmogorov-Smirnov test; comparison of quantitative variables by ANOVA followed by Bonferroni's post hoc test and student *t*-test. Chi-square analysis and correlation coefficient r calculated to measure the association between MYPT-1 phosphorylation state and LV mass.

Results

MYPT-1 phosphorylation state in dialysis, stage 3-4 CKD patients and healthy controls

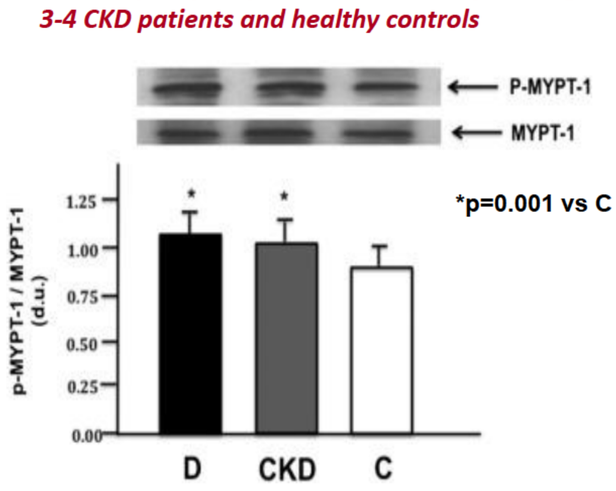


Fig. 1. Densitometric analysis of phospho-MYPT-1 to MYPT ratio in mononuclear cells of dialysis patients (D, black column, n = 23), stage 3 and 4 CKD patients (CKD, grey column, n = 13) and healthy subjects (C, white column, n = 30). The top of the figure shows representative phosphorylated phospho-MYPT-1 western blot products from 1 D pateint, 1 CKD and 1 C.

Effect of ROCK inhibition with fasudil on MYPT-1 phoshorylation

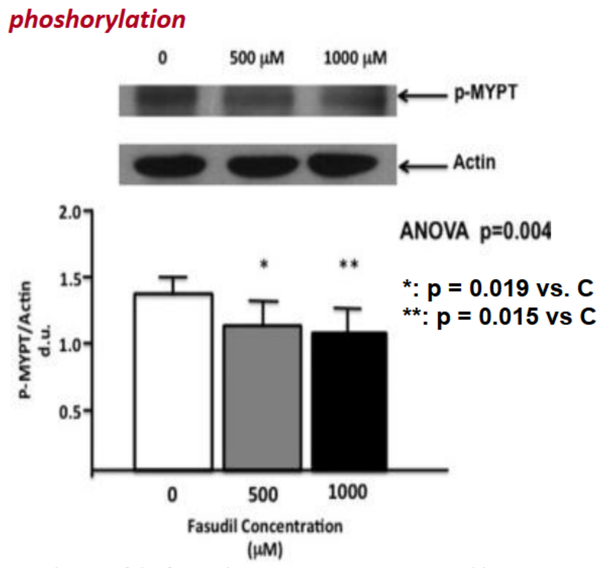


Fig. 2. The top of the figure shows representative Western blot products from 1 dialysis patient.

MLVH and MYPT-1 phosphorylation reltionship

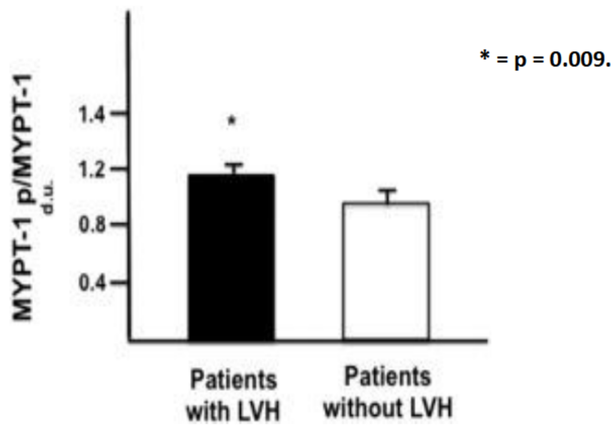
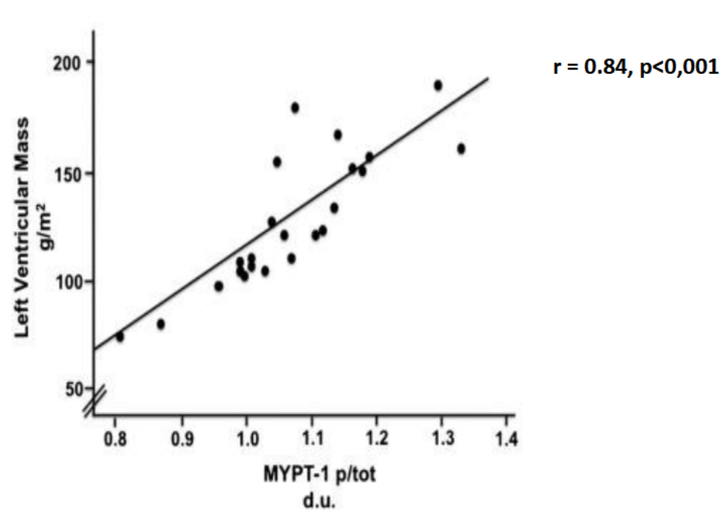
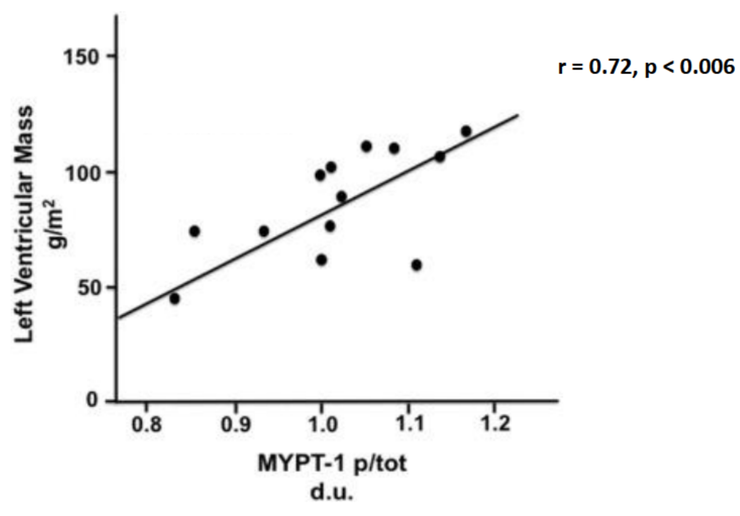


Fig. 3. MYPT-1 phosphorylation levels in dialysis and stage 3–4 CKD patients with (n = 19) and without (n = 17) LVH.

Correlation between MYPT-1 phosphorylation and cardiac LV mass in dialysis patients



Correlation between MYPT-1 phosphorylation and cardiac LV mass in stage 3–4 CKD patients.



Conclusions

The RhoA/ROCK system plays significant roles in both the regulation of blood pressure as well as vascular smooth muscle tone [7]. Upon activation by Ang II coupling with its receptor AT1R , RhoA translocates to the cell membrane and activates its target ROCK. which in turn phosphorylates MYPT-1 inhibiting its activity. ROCK, via an inhibitory phosphorylation of MYPT-1, increases the activity of myosin light chain kinase, leading to smooth muscle contraction and cardiovascular and renal remodeling [8].

The current study in dialysis and stage 3–4 CKD patients, a category of very high risk patients for cardiovascular morbidity and mortality, shows that ROCK activity in circulating leukocyte is not only higher in dialysis and stage 3–4 CKD patients compared to healthy subjects but is also higher in dialysis and stage 3–4 CKD patients with LVH compared to those without LVH. Moreover, we provided a demonstration of higher ROCK activity in the dialysis and stage 3–4 CKD patients of our study by showing the effect of ROCK activity inhibition with fasudil. MYPT-1 phosphorylation was, in fact, dose dependently and significantly reduced by the incubation of patients' mononuclear cells with the ROCK inhibitor fasudil, indicating that in humans, ROCK activation in mononuclear cells causes MYPT-1 phosphorylation. Thus, both our results in dialysis/CKD patients and those in hypertensive patients [3] show that elevated ROCK activity is associated with cardiac remodeling in these two different groups of high risk patients for cardiovascular morbidity and mortality.

The current study provides evidence linking the activation of ROCK, as reflected by the increased mononuclear cell phosphorylation state of MYPT-1, to cardiac hypertrophy in dialysis and stage 3-4 CKD patients. The results of this study join those showed in another high risk for cardiovascular disease patients such as hypertensive patients and receive indirect support from data provided by a human clinical condition opposite to hypertension and type 2 diabetes (T2D) identifying ROCK activation as a potential LVH marker. Finally, these data provide further rationale for ROCK activation inhibition as target of therapy in patients at high risk for cardiovascular disease such as CKD, dialysis, hypertensive and T2D patients.

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