Journal of

Cardiology and Therapy

Online Submissions: http://www.ghrnet.org/index./jct/
doi:10.17554/j.issn.2309-6861.2015.02.102

Journal of Cardiol Ther 2015 December 2(6): 465-468
ISSN 2309-6861(print), ISSN 2312-122X(online)

REVIEW

The Isoform Spesific Roles of Rho-Kinases in Vascular Diseases

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Received: July 23, 2015 Revised: November 28, 2015
Accepted: December 2, 2015
Published online: December 10, 2015

ABSTRACT

A small GTPase RhoA and its downstream effectors Rho-associated protein kinases (ROCK) signaling pathway activation mediate smooth muscle contraction. ROCKs inhibit myosin light chain phosphatase (MLCP) dephosphorylation and therefore reduce relaxation. However, nitric oxide (NO) that is produced and released from endothelial cells has an inhibitory effect on the ROCK pathway in vasculature. Studies in which ROCK activity was inhibited by variety of pharmacological agents (HA1077 or Y-27632) have shown that it has some critical effects on systemic diseases like hypertension or diabetes mellitus. Indeed this activity may show isoform specificity (ROCK1 or ROCK2) dependent on the pathology. Therefore, in vascular pathogenesis ROCK pathway with its isoforms also need to be considered due to its direct effects on the vasoconstriction.

Key words: Rho Kinases; ROCK1; ROCK2; Vasoconstriction; Vascular diseases

Çiçek F, Ayaz M. The Isoform Spesific Roles of Rho-Kinases in Vascular Diseases. *Journal of Cardiology and Therapy* 2015; 2(6): 465-468 Available from: URL: http://www.ghrnet.org/index.php/jct/article/view/1517

INTRODUCTION

Rho-associated protein kinases (ROCKs) play a critical role in smooth muscle contraction and relaxation. After activation of small GTPase RhoA its effector protein ROCK mediates contraction. Briefly after a signal arrives to cell membrane and activates membrane receptors or voltage operated Ca^{2+} channels (VOCC) free cytoplasmic Ca^{2+} concentration increases. Then Ca^{2+} binds to calmodulin and activates myosin light chain kinase (MLCK). MLCK phosphorylates myosin light chains (MLC), which are the regulatory subunits of the myosin heads.

MLCK phosphorylates (MLC) the subunits of the myosin heads. Phosphorylated MLCs enable the cross bridges between myosin and actin and so contraction occurs in smooth muscles[1,2]. On the other hand RhoA mediated ROCK is the other mediator of contraction with Ca^{2+}[3]. Once activated ROCK provides continuation of contractile activity by inhibiting MLC phosphatase (MLCP) which dephosphorylate MLC and induces relaxation in smooth muscle cells. Together with Ca^{2+}, ROCK pathways precisely control the vasoconstriction. In the arteries smooth muscle contraction is directly effects blood pressure by regulating the vessel diameter and tension[1,4]. In addition to vasoconstrictor effectors, endothelial derived Nitric Oxide (NO) is a vasodilator agent for smooth muscle cells which regulates the relaxation through cGMP pathway and also reduces ROCK activity and thereby contraction[5-8] (Figure 1). In the regulation of the vascular tonus these vasoconstrictor and vasodilator pathways mediate contraction. In recent years many studies has shown that Rho Kinase pathway should be taken into consideration in treatments of vascular diseases[9,10].

ROCK consists of two isoforms ROCK1 and ROCK2. ROCK1 enzyme is expressed in a plenty of different tissues like lung, kidney, stomach whereas ROCK2 is mostly expressed in heart, brain and skeletal muscle[11]. Cellular localization of the ROCK1 and ROCK2 also show diversity. ROCK1 is mostly localized at plasma membrane but ROCK2 at centrosomes of smooth muscle cells. At cardiomyocytes ROCK2 localized at intercalated discs, and at skeletal muscle cells Z-discs and sarcoplasmic reticulum[12].

Although, they have high genetic homology in their kinase domain

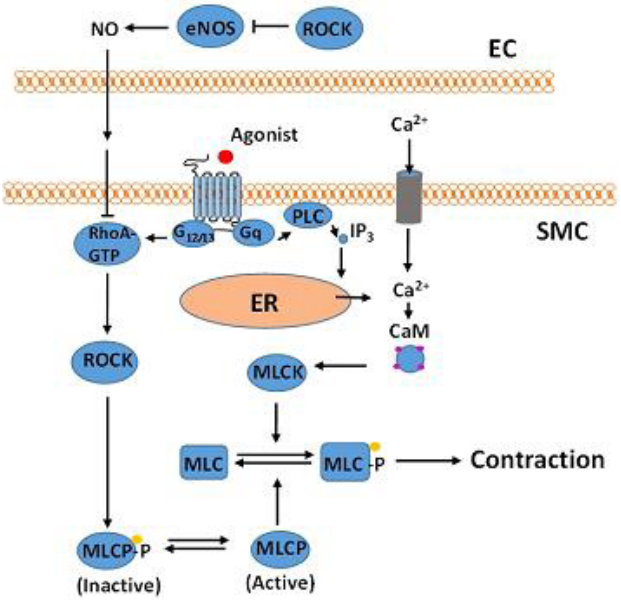

Table 1 The contribution of ROCK pathway onto the contraction of smooth muscle. ROCK enhance contraction by phosphorylating MLCP. This inactive form of MLCP could not dephosphorylate MLC which leads to attenuation of the muscle relaxation. ROCK activity is reduced with the NO through cGMP pathway. Abbreviations: PLC: Phospholipase C, IP3: Inositol (1,4,5)-trisphosphate, eNOS: Endothelial nitric oxide synthase, MLC: Myosin light chains, MLCK: MLC kinase, MLCP: MLC phosphatase, ROCK: Rho kinase, NO: Nitric oxide.

(92%)[13], Yoneda *et al*[14] (2005) showed that they have isoform specific functions even in the same cell. According to this study ROCK1 is more active in focal adhesion and fiber formations rather than ROCK2 in primary rat embryo fibroblasts. Conversely, ROCK2 is the primer player in phagocytic activity. In another study it was shown that ROCK1 and ROCK2 have distinct roles in adhesion and differentiation in keratinocytes[15]. Also the experiments performed with the cells which derived from ROCK1 and 2 knockout animals, was shown that ROCK 1 acts in MLC2 phosphorylation and cell detachment, whereas ROCK2 in actin cytoskeleton stabilization[16]. Their mechanistic difference was indicated by Wang Y *et al*[17] (2009) that ROCK2 can bind directly to the myosin-binding subunit (MYPT1) of myosin phosphatase but not ROCK1. This difference reflects that ROCK1 use intermediate components for MLCP inhibition and this fringed pathway selection makes harder to understand the ROCK activity.

However lack of the isoform specific ROCK blockers (HA1077 and Y27632 are nonspecific blockers of ROCK) make difficult to distinguish the functional roles of the isoforms. But such a critical pathway that regulates constrictive mechanisms in vascular system deserves more precise evaluation. Therefore in this review we tried to we focus on these functional differences between two isoforms in vasculature from diseases perspective.

ROCK ISOFORMS IN ARTERIAL AND PULMONARY ARTERIAL HYPERTENSION

Smooth muscle cells primarily regulate vascular volume and thereby blood pressure in the aorta. The role of the vasoconstrictors on the hypertension is a detailed examined in many studies concluded ROCK also play a major role in persistency of the high pressure in aorta[2,18].

It was shown in the hypertensive animal models that ROCK mediated vasoconstriction is involved arterial hypertension by blocking its function with Y-27632[19]. Other selective ROCK inhibitor HA1077 named as fasudil is believed to be a key therapeutic for human use. In one study dealing with hypertensive patients it was shown that the fasudil induce a vasodilator effect on the arterial pressure[20]. Also in a study Fukumoto *et al*[21] showed the effects of the fasudil on the patients with pulmonary arterial (PA) hypertension. The treatment with fasudil hydrochloride caused a slight decrease in the PA hypertension.

In both of these arterial high pressure diseases differences in the expression levels of ROCK1 and ROCK2 were observed. The immunostaining experiments ROCK2 (but not ROCK1) showed that its expression increases in arterioles of the lung sections taken from the PA hypertension patients[22]. The same study indicated that the hypoxia induced PA hypertension with vascular smooth muscle specific ROCK2 gene knockout mice, the right ventricular systolic pressure was significantly reduced versus control. Their findings indicate the importance of ROCK2 for the development of hypoxia-induced PA hypertension. Also ROCK2 gene silencing was improved erectile function on spontaneously hypertensive rats suggesting ROCK2 inhibition can be used as a specific therapeutic target for vascular dysfunctions caused by hypertension[23].

ATHEROSCLEROSIS

When dealing with this very complicated inflammatory disease we see that on tunica intima, the layer surrounded with the formations such foam cells (monocytes/macrophages) that decrease vessel diameter and even make it more stiffening[24,25]. Impaired endothelium activity (endothelium dysfunction) causes dysregulation of NO release, which was thought as a major responsible factor for the initiation of atherosclerosis[26,27]. According to the study of Anju Nohria *et al*[28] ROCK inhibition with fasudil caused endothelial dependent vasodilation in the patients with coronary artery disease. Their measurements with brachial artery ultrasonography suggest the relation between endothelium activity and ROCK inhibition in atherosclerosis. In another study with mice ROCK inhibition with Y27632 results a protection against atherosclerosis by reducing significantly size of the atherosclerotic plaque formation significantly[29].

The individual roles of ROCK1 and ROCK2 in atherosclerosis tried to be explained in several studies. ROCK1 knockout was decreased atherosclerotic lesion formations in aortas from the bone marrow (BM) derived macrophage transplanted LDLr knockout mice[30]. While the experiments with ROCK2 lacking in the cultured BM differentiated macrophages was shown the importance of ROCK2 in the foam cell formations[31].

DIABETES

Type independently, diabetes mellitus (DM) patients frequently suffer from the complications of circulatory system diseases such as cardiovascular or other vascular diseases. These complications may accompany with hypertension, atherosclerosis and thereby some ischemic diseases or systemic dysfunctions (peripheral, pulmonary, renin-angiotensin)[32-35]. It was shown that Rho kinase has a promoter effect on Ca^{2+} sensitive vasocontraction with PKC in STZ induced DM model studies[36]. Also in the study by Sandu OA *et al*[37] (2001) the interaction of insulin with Rho kinase from phosphatidylinositol 3-kinase (PI3-kinase) and iNOS activated NO-cGMP pathway was specified in vascular smooth muscle cells (VSMC). According to them insulin receptor activation inhibits ROCK activity by

the NO pathway and a defectiveness in this pathway in diabetes and hypertension may lead an impaired relaxation with increased ROCK activity and resulting vasoconstriction. Also from the Rho-kinase activity experiments it was observed that arteries from Zucker diabetic fatty (ZDF) rats or incubated with high glucose concentrations, ROCK activity increase parallel with the glucose concentration[38]. Rikiteka *et al* (2005) also has shown the correlation between vascular endothelial cells (HSVECs) and ROCK activity that increases in high glucose[39]. In the same study the high levels of Plasminogen activator inhibitor-1 (PAI-1) protein expression induced with hyperglycemia decreased in ROCK1 knockout (ROCK $I^{+/-}$) murine lung endothelial cells. While PAI-1 is a risk factor in many vascular diseases[40], the effect of ROCK on the expression of this protein in hyperglycemia will also show the key role of ROCK activity in vascular dysfunctions.

In DM induced circulatory system diseases endothelial dysfunction which led impaired NO bioavailability causes impaired vasodilation. Many study show the effect of the ROCK pathway on the endothelial dysfunction and which then leads to impaired relaxation. In DM induced vascular endothelial dysfunction (VED) Rho kinase inhibition with fasudil improved eNOS/NO dependent vasodilation is stimulated by acetylcholine[41]. Also in diabetic retinopathy, a microvascular endothelial dysfunction, it was found that high glucose concentration has increased ROCK activity in retinal endothelial cell line, RF/6A cells[42].

There are also ROCK1 and ROCK2 isoform specific studies in DM. Yao L (2013) by partly deletion both isoforms showed that ROCK1 is more effective in diabetic mice aorta according to vasorelaxive response to acetylcholine[43]. However in endothelial cells of rat thoracic aorta ROCK2 protein expression was found higher in DM with respect to the control group[44]. This difference may reflect different functional properties of ROCKs in the regulation of vascular smooth muscle contractions in DM.

Overall ROCK is a key player of many cellular functions. In recent years growing studies elicited its role in regulation of blood pressure in the vessels and therefore should be considered along with other contraction parameters. The isoforms ROCK1 and ROCK2 show branched functions, and regulate many diverse cellular activities on the circulatory system cells. Therefore, particularly in the treatment of cardiovascular diseases ROCKs with their isoforms should be taken into consideration because of their direct interventions on vasoconstriction

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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