The Isoform Specific Roles of Rho-Kinases in Vascular Diseases

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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.

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

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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 dephosphorylates 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
It was shown in the hypertensive animal models that ROCK medi-
ate vasoconstriction is involved arterial hypertension by blocking its
function with Y-27632\textsuperscript{[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\textsuperscript{[20]}. Also in
a study Fukumoto \textit{et al}\textsuperscript{[21]} showed the effects of the fasudil on the pa-
tients 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 im-
munostaining experiments ROCK2 (but not ROCK1) showed that
its expression increases in arteries of the lung sections taken from
the PA hypertension patients\textsuperscript{[22]}. The same study indicated that the hy-
poxia 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\textsuperscript{[23]}.

\section*{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\textsuperscript{[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\textsuperscript{[26,27]}. According to the study of Anju
Nohria \textit{et al}\textsuperscript{[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\textsuperscript{[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\textsuperscript{[30]}. While the experiments with ROCK2 lacking in the cultured
BM differentiated macrophages was shown the importance of
ROCK2 in the foam cell formations\textsuperscript{[31]}.

\section*{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)\textsuperscript{[22,23]}. It was shown that Rho kinase has a promoter
effect on Ca\textsuperscript{2+} sensitive vasocontraction with PKC in STZ induced
DM model studies\textsuperscript{[24]}. Also in the study by Sandu \textit{et al}\textsuperscript{[25]} (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 diabetic complications 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. Rikitake et al. (2005) also has shown the correlation between vascular endothelial cells (HSVECs) and ROCK activity which increases in high glucose. In the same study the high levels of Plasminogen activator inhibitor-1 (PAI-1) protein expression induced with hyperglycemia decreased in ROCK I knockout (ROCK I−/−) murine lung endothelial cells. While PAI-1 is a risk factor in many vascular diseases, 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. 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. 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. However in endothelial cells of rat thoracic aorta ROCK2 protein expression was found higher in DM with respect to the control group. 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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