

CoTherix, Inc.
Fasudil, Rho-kinase Inhibitor
June 26, 2006

Fasudil: A Direct Rho-kinase Inhibitor

Fasudil is an orally bioavailable rho-kinase inhibitor discovered by Asahi Kasei. It is a small molecule and is currently the only rho-kinase inhibitor in clinical trial use. Fasudil was first developed by Asahi Kasei for the prevention of cerebral vasospasm in patients with subarachnoid hemorrhage. Asahi Kasei received Japanese approval for the intravenous formulation in 1995.

Fasudil directly inhibits rho-kinase and has shown promise as a treatment for stable angina and pulmonary hypertension. Pre-clinical research over the past decade has identified rho-kinase as an important therapeutic target in several cardiovascular and pulmonary disease models including atherosclerosis, hypertension, restenosis and asthma.

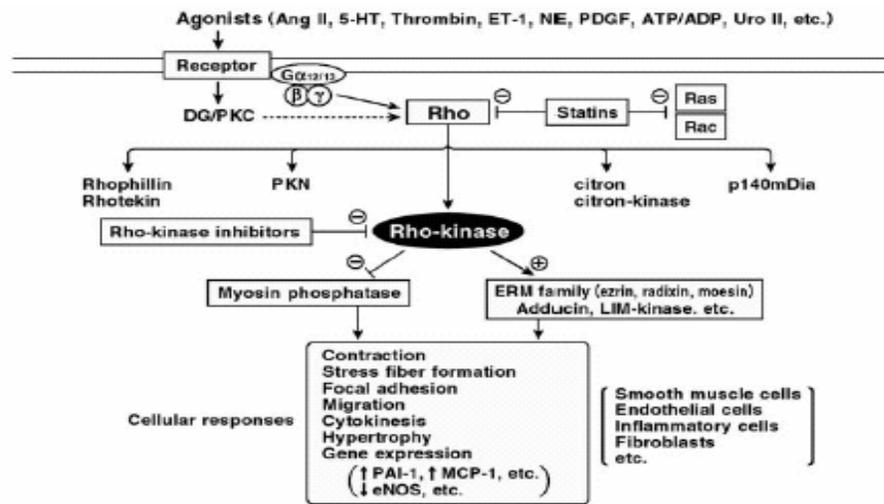
Rho GTPase is a low molecular weight G protein that acts as an intracellular switch between an inactive GDP-bound state and an active GTP-bound state to regulate cellular functions. Rho is the term generally used to describe the rho subfamily consisting of 3 human homologs: rhoA, rhoB and rhoC. Several proteins are activated by rho but the best characterized is rho-kinase (consisting of two isoforms also known as ROCK α and ROCK β). Rho and rho-kinase proteins are involved in a variety of biochemical signalling pathways in cells and have important functions in vascular physiology and pathophysiology.

Rho/Rho-kinase in Cardiovascular Disease

Pre-clinical studies and emerging clinical evidence have demonstrated that the rho/rho-kinase pathway (Rho/ROCK) plays an important role in regulating and balancing several aspects of cellular function that are involved in the pathogenesis of cardiovascular and pulmonary vascular diseases. For example, Rho/ROCK is highly activated under conditions of inflammation and injury. Various types of inflammatory stimuli can activate Rho/ROCK signalling causing the pathway to 1) upregulate cytokines & inflammatory cells that further increase inflammation and 2) downregulate endothelial nitric oxide synthase (eNOS).

ROCK has a major effect on the control of tone in vascular smooth muscle (VSM). Excess activation of ROCK leads to sustained hypercontraction of VSM (sustained abnormal vasoconstriction). Pre-clinical studies in models of atherosclerosis have shown that increased Rho/ROCK activity also promotes vascular inflammation. Atherosclerotic plaque in the coronary arteries, which causes narrowing of the lumen, is a common underlying cause of stable angina.

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Source: Shimokawa, *Journal of Cardiovascular Pharmacology*: Vol. 39, no.3, 2002¹

In addition to upregulating pro-inflammatory cells and cytokines, ROCK activates a number of pathways that induce thrombosis and cell proliferation and/or hypertrophy. Models of pulmonary vascular and cardiovascular diseases have shown that inhibition of ROCK attenuates or reverses perivascular inflammation and vascular smooth muscle cell remodeling (thickening of the vessel wall). The beneficial effects of ROCK inhibition have also been demonstrated in the myocardium by the reduction of hypertrophy in heart failure models.

Inflammation, Vasoconstriction & Remodeling in Vascular Smooth Muscle

Some of the known mediators of inflammation that initiate signal transduction of the Rho/ROCK pathway include endothelin-1, angiotensin II, serotonin, thrombin, thromboxane and platelet derived growth factor (PDGF). These agonists are activated in pulmonary hypertension and several other cardiovascular disease states, and are associated with functional and structural vascular changes.

The primary mechanism of VSM cell contraction is regulated by increases in intracellular calcium (Ca²⁺). Increased intracellular Ca²⁺ activates myosin light chain kinase, which initiates muscle contraction by stimulating phosphorylation of myosin light chains (MLC). Another important mechanism that contributes to VSM cell contraction can occur independently of increases in cytosolic Ca²⁺ and is known as Ca²⁺ sensitization.

Ca²⁺ sensitization has been linked to the inhibition of myosin light chain phosphatase by ROCK. The inhibition of myosin light chain phosphatase is a key step that leads to an accumulation of phosphorylated MLC which results in an increase in vascular tone (vasoconstriction). VSM cell hypertrophy and/or proliferation are responses to long-term vasoconstriction.

¹ H Shimokawa, Rho-kinase as a Novel Therapeutic Target in Treatment of Cardiovascular Diseases. *Journal of Cardiovascular Pharmacology*. 2005: Vol 39:319-327

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The endothelium maintains vascular homeostasis and integrity, and nitric oxide (NO) is a key signaling molecule that mediates many of these protective functions. NO improves vasorelaxation and decreases inflammatory cell accumulation at the vessel wall. Endothelial NO synthase (eNOS) is responsible for producing NO in the endothelium. Reduced eNOS has been implicated in the pathophysiology of pulmonary hypertension and other cardiovascular diseases. Pre-clinical models of pulmonary hypertension and atherosclerosis have shown that the Rho/ROCK pathway downregulates eNOS expression, which can be counteracted by inhibition of ROCK.

Diseased vessels in pulmonary hypertension and coronary artery disease show visible changes in their structural features. Endothelial dysfunction has a central role in the initiation and progression of disease. Changes in the endothelium, including increased permeability and expression of adhesion molecules, initiate an inflammatory response that promotes cell migration, proliferation and hypertrophy. Pre-clinical studies have provided evidence that the inhibition of Rho/ROCK attenuates accumulation of inflammatory cells and VSM cell migration, hypertrophy and proliferation induced by angiotensin II, thrombin and PDGF.

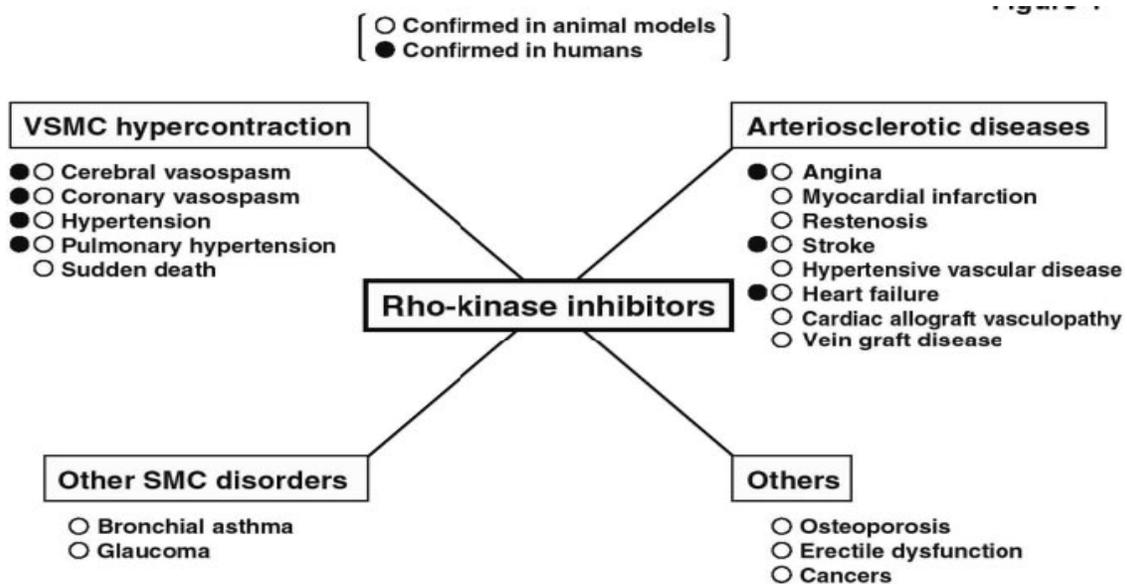
Fasudil: A Promising Mechanism of Action in Cardiovascular Disease

Fasudil directly inhibits ROCK, which is a well characterized target implicated in vascular disease. Fasudil has vasodilating properties and attenuates inflammation, cell migration and proliferation in disease models. CoTherix plans to perform trials to explore the clinical implications of treatment with fasudil. In particular, CoTherix intends to develop an extended release oral formulation of fasudil with a goal of enabling twice-daily dosing and to provide a smoother pharmacokinetic profile. The Company also intends to explore the development of inhaled fasudil for pulmonary hypertension.

In addition to treating patients with stable angina and pulmonary arterial hypertension (PAH), fasudil has the potential to treat a number of diseases and conditions including the following: (also see Figure below)

- *Atherosclerosis.* Rho/ROCK activation has been identified as a contributing factor in experimental models of vascular inflammation. Inhibition of ROCK has been associated with a reduction of early atherosclerotic lesion formation in pre-clinical studies. Angiotensin II, in addition to increasing blood pressure, promotes vascular inflammation and appears to have a central role in the pathophysiology of atherosclerosis. Fasudil was shown in an experimental model of apolipoprotein E-deficient mice (apoE-KO) to attenuate Ang II-induced abdominal aortic aneurysm formation by inhibiting apoptosis and proteolysis. Inhibiting ROCK activation in atherosclerosis may provide a clinical benefit.
- *Hypertension and hypertensive cardiac hypertrophy.* ROCK activity has also been investigated in the pathogenesis of hypertensive vascular disease. Some of the cellular processes that influence vascular resistance include abnormal vasoconstriction, VSM cell proliferation and hypertrophy, and inflammatory cell adhesion. ROCK is a known mediator of the pathways that regulate these processes, as well as a regulator of smooth muscle contraction. Short term administration of a ROCK inhibitor decreased blood pressure in several animal models of systemic hypertension. In another experimental model, long term administration of fasudil inhibited angiotensin II-induced cardiac hypertrophy. Also, non-hypotensive doses of fasudil have been shown to suppress coronary vascular lesion formation in spontaneously hypertensive rats.

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Source: Shimokawa, *Journal of Cardiovascular Pharmacology*: Vol. 39, no.3, 2002

Japanese investigators conducted two trials of an immediate release oral formulation for the treatment of stable angina in Japan and, in August 2001, Schering AG in-licensed the oral formulation of fasudil from Asahi Kasei with rights to develop and market the product in the U.S. and Europe for stable angina. In August 2002, Schering AG in-licensed the intravenous formulation of the drug with marketing rights in the U.S. and Europe. Schering AG subsequently made a corporate decision that cardiovascular disease would no longer represent a core business field, divested its cardiovascular assets and returned the rights to develop fasudil to Asahi Kasei in late 2005.

Fasudil: Clinical Proof-of-Concept in Pulmonary Hypertension

Pre-clinical studies have reported that Rho/ROCK signalling is involved in increased vasoconstriction and remodeling in models of pulmonary hypertension. Furthermore, it has been shown that inhibition of ROCK attenuated or reversed these effects. This preliminary work led two separate physician investigator groups in Japan to examine the acute effects of intravenous fasudil on the hemodynamics of patients with PAH. These trials have provided early proof-of-concept that single intravenous infusions of fasudil could provide a beneficial acute hemodynamic response in pulmonary hypertension.

- *Intravenous fasudil study conducted by Fukumoto, et al in Japan (Investigator-sponsored trial at Kyushu University Hospital).* In a single center, nine patients with PAH (six female and three male) were treated acutely with intravenous fasudil. Results from the trial were published in 2005.²

² Y Fukumoto, T Matoba, A Ito, H Tanaka, T Kishi, S Hayashidani, K Abe, A Takeshita, H Shimokawa. Acute Vasodilator Effects of a Rho-kinase Inhibitor, fasudil, in Patients with Severe pulmonary Hypertension. *Heart* 2005; 91:391-392

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- Patients were allowed to continue all medications including oral or intravenous prostacyclin. A Swan-Ganz catheter was inserted to measure pulmonary hemodynamics.
- Patients received 30mg of fasudil over 30 minutes. Hemodynamic measurements were reported over a 60 minute period.

After treatment, the patients had a slightly decreased mean pulmonary arterial pressure (PAP) and a small increase in cardiac index (CI) compared to baseline. However, the treatment caused a significant reduction of 17% in pulmonary vascular resistance (PVR). The investigators reported no side effects.

- *Intravenous fasudil study conducted by Ishikura, et al in Japan (Investigator-sponsored trial at Mie University Hospital).* In a single center, eight patients with PAH (all female) were treated acutely with intravenous fasudil. Five patients had idiopathic PAH (IPAH) and three had PAH associated with collagen vascular disease (CPAH). Four patients were diagnosed with PAH within two months prior to trial enrollment and were not on any treatment. Results from the trial were published in 2006.³
 - No vasodilator was given for at least 12 hours before administration of fasudil. A Swan-Ganz catheter was inserted to measure pulmonary hemodynamics.
 - Patients received 1mg/min of fasudil for 30 minutes. Hemodynamic monitoring was performed continuously and recorded for up to 60 minutes after administration.

After treatment with fasudil, patients in this trial had a significant decrease in total pulmonary resistance (TPR) of -23.9% of baseline ($p < 0.005$), a significant decrease in PAP of $-9.9 \pm 10.7\%$ of baseline ($p < 0.05$), and a significant increase in CI of $19.4 \pm 15.5\%$ of baseline ($p < 0.02$). There was no significant change in oxygen saturation which can occur with systemic vasodilators. No major side effects were reported.

Fasudil: Clinical Proof-of-Concept in Chronic Stable Angina

ROCK plays a major role in Ca^{2+} sensitization of VSM and appears to be involved in the pathogenesis of coronary vasospasm and angina. Inhibition of ROCK with fasudil causes vasodilation of the vessels.

Shimokawa has published several dose escalation trials of fasudil in stable angina, which provide the first proof-of-concept in humans. Details of these trials are provided below.⁴

- *Oral study conducted by Shimokawa, et al in Japan (Phase IIa).* In a Phase IIa multi-center (23 center) study, 67 patients with stable exercise-induced angina pectoris were treated with two different ascending dose regimens of fasudil. A 2-week placebo run-in period preceded treatment in both parts of the study.

³ K Ishikura, N Yamada, M Ito, S Ota, M Nakamura, N Isaka, T Nakano. Beneficial Acute Effects of Rho-Kinase Inhibitor in Patients With Pulmonary Arterial Hypertension. *Circulation Journal*. Feb. 2006. Vol 70: 174-178.

⁴ H Shimokawa, K Hiramori, H Iinuma, S Hosoda, H Kishida, H Osada, T Katagiri, K Yamauchi, Y Yui, T Minamino, M Nakashima, K Kato. Anti-anginal Effect of Fasudil, a Rho-Kinase Inhibitor, in Patients With Stable Effort Angina: A Multicenter Study. *Journal of Pharmacology*. 2002 40:751-761.

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In the first part of the study, 45 patients were treated with increasing doses of fasudil at 15, 30, and 60 mg/day, t.i.d, administered sequentially for 2-week periods until anginal attacks resolved. In this part of the study, it was shown that fasudil significantly decreased the number of anginal attacks per week ($p<0.001$) and significantly prolonged the maximum exercise time ($p<0.01$), as well as the time to the onset of 1-mm ST segment depression.

In the second part of the study, 22 patients were treated with fasudil at 60 mg/day for 2 weeks, then 120 mg/day, t.i.d., for 2 weeks. In this part of the study, the number of angina attacks was significantly reduced ($p<0.001$), the use of sublingual nitroglycerine significantly decreased ($p<0.05$) and the maximum exercise time was significantly prolonged ($p<0.05$).

Oral study conducted by Shimokawa et al in Japan (Phase IIb). In a Phase IIb multi-center (46 center) study, 125 patients with stable exercise-induced angina pectoris were randomized to receive fasudil 15, 30, 60, or 120 mg/day, t.i.d., for four weeks.

Although all exercise parameters (1) total exercise duration, 2) time to onset of 1-mm ST segment depression, and 3) ST segment depression at the same time) were prolonged during fasudil treatment, there was no apparent dose-response relationship in terms of the effects of fasudil on the individual parameters. However, when the three indices were combined as an exercise tolerance index, a highly significant dose-response relationship was observed ($p=0.006$), which best fit a linear relationship. No dose-response relationship was observed with respect to any of the other investigated parameters. However, the number of angina attacks significantly decreased in all groups although there was no significant dose-dependent decrease in the number of anginal attacks across the four groups.

Oral study conducted by Berlex (Schering AG) (Phase II). In the Phase II randomized double-blind, placebo controlled study conducted by Schering AG, 84 patients with stable angina pectoris were to receive oral fasudil or placebo (41 fasudil, 43 placebo). Doses of fasudil were increased every two weeks for eight weeks to the highest dose of 240 mg/day administered in three divided doses. Patients were allowed to take one anti-anginal medication and short-acting nitrates as needed, along with their usual cardiovascular medications including aspirin and statins.⁵

- Results showed that exercise duration at eight weeks was increased by 1.43 minutes in the placebo group and by 1.97 minutes in the fasudil group.

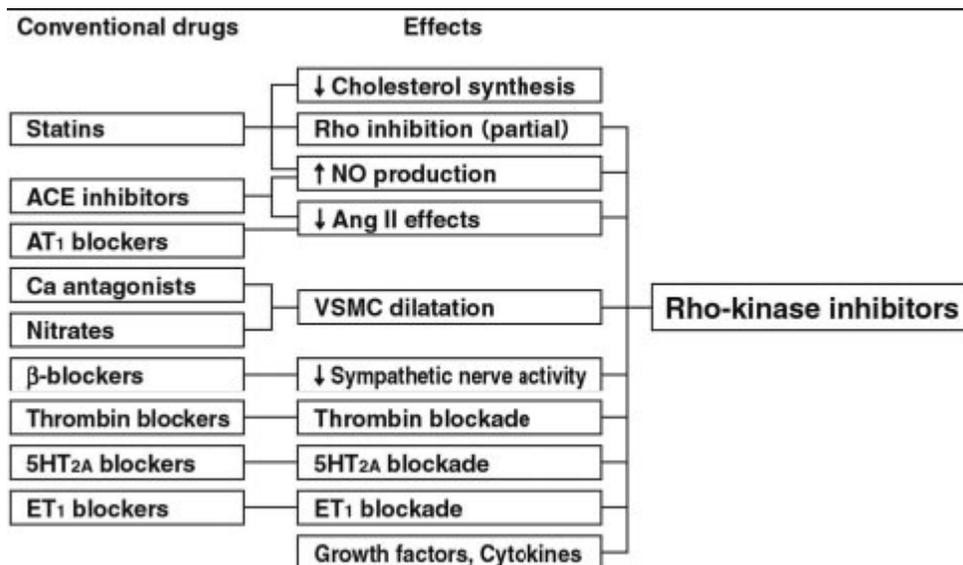
Time to 1-mm ST segment depression (onset of myocardial ischemia) was increased by 2.83 minutes in the fasudil group compared to placebo at eight weeks ($p=0.012$). Fasudil was well tolerated in patients with stable exercise-induced angina pectoris.

CoTherix Development Activities Summary

CoTherix intends to develop fasudil for the treatment of PAH and stable angina. Fasudil is the only ROCK inhibitor that has been tested in the clinic and, if approved, it would be a first-in-class drug. Rho/ROCK proteins have important functions in vascular physiology and pathophysiology. Pre-clinical studies have shown repeatedly that the inhibition of ROCK has promise as a treatment for both pulmonary vascular and cardiovascular diseases.

⁵ R Vicari, B Chaitman, D Keefe, W Smith, S Chrysant, M Tonkon, N Bittar, R Weiss, H Morales-Ballejo, U Thadani. Efficacy and Safety of Fasudil in Patients with Stable Angina, A Double-Blind, Placebo-Controlled, Phase 2 Trial. *Journal of American College of Cardiology*. 2005 46:10

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Source: H Shimokawa, *Arteriosclerosis Thombosis Vascular Biology* : Sept 2005 ⁶

The inhibition of ROCK has broad pharmacological implications and has the possibility of complementing the therapeutic activities of a number of different drugs (see Figure above). The addition of fasudil to CoTherix's pipeline enables the Company to expand its footprint in PAH and to enter into the cardiovascular market. The encouraging Phase II data for oral fasudil in stable angina, the promising proof-of-concept trial results in PAH patients, the potential to apply it to other diseases and its stage of development provides CoTherix with a promising clinical stage drug.

Forward-looking Statements

This document includes forward-looking statements regarding CoTherix's expectations, beliefs, hopes, goals, plans, intentions, initiatives or strategies, including but not limited to statements about the therapeutic potential of Fasudil for PAH, stable angina and other indications; the promising nature of Fasudil and its mechanism of action; significance of the Rho/Rhokinase pathway; CoTherix's development plans; expectations that fasudil, if approved, would be a first-in-class drug; CoTherix's potential and ability to expand its footprint in PAH, enter into the cardiovascular market and add to its pipeline; and the development of other compounds. All forward-looking statements included in this presentation are based on information available to CoTherix as of the date hereof, and CoTherix assumes no obligation to update any such forward-looking statement as a result of new information, future events or otherwise. CoTherix's actual results and other events could differ materially from its expectations. Factors that could cause or contribute to such differences include, among other things, development and approval of the use of Fasudil for various indications; patient market size and market adoption by physicians and patients; difficulties and delays in developing, testing, obtaining regulatory approval of, and producing and marketing Fasudil; competition from other companies; and other factors discussed in the "Risk Factors" section of CoTherix's most current Quarterly Report on Form 10-Q and other reports filed with the SEC.

⁶ H Shimokawa, A Takeshita. Rho-Kinase Is an Important Therapeutic Target in Cardiovascular Medicine. *Arteriosclerosis Thombosis Vascular Biology*. Sept 2005