Statin-Mediated Inhibition of Rho
Only to Get More NO?

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HMG-CoA reductase inhibitors (statins) are more than just cholesterol-lowering drugs. The effects of statins in the cardiovascular system include, among others, the improvement of endothelium-dependent relaxation, a reduction in the progression of arteriosclerosis, an antihypertensive effect and beneficial actions on cardiac function. The pleiotropic actions even extend beyond the cardiovascular system and have been suggested to attenuate the progression of osteoporosis, reduce the incidence or severity of dementia, type II diabetes, allograft organ rejection and inflammation. Some of the effects of statins are attributed to interactions of the drugs with cell surface receptors, effects on cytochrome P450 monoxygenases and actions on the PI3-kinase pathway. The most important mechanism for the pleiotropic effects, however, is the inhibition of small GTPases. Small GTPases, which include proteins of the Ras and Rho families, mediate essential cellular signals required for proliferation, migration, and gene expression.

To elicit their biological effects, small GTPases have to be anchored in the plasma membrane by an isoprenoid tail. Isoprenoids are intermediates of the cholesterol de novo synthesis. By inhibiting the key enzyme of the cholesterol de novo synthesis, the HMG-CoA reductase, statins deplete the cell of these lipids and thus elicit the retention of small GTPases in the cytosol, where they cannot exert their biological actions.

One of the best examined consequences of a statin-mediated inhibition of small GTPases is a rapid improvement of endothelial function, which is thought to be mediated by 2 different mechanisms: the inhibition of Rac, a member of the Rho family of small GTPases, prevents the activation of NADPH oxidases and reduces vascular oxidative stress. Probably more important, inhibition of RhoA, another member of the Rho family, stabilizes the mRNA of the endothelial NO synthase (eNOS), and thus increases endothelial eNOS protein level.

In this issue of Circulation Research, Shiga et al provide further evidence that RhoA activity is a critical determinant for eNOS protein expression in isolated strips of the rabbits mesenteric artery in organ culture and in vivo. To demonstrate this, the authors used recombinant signaling-inactive proteins of the RhoA-binding domain of Rho-kinase and of the Rac1/Cdc42-binding domain (PBD) of p21-activated kinase-1 (PAK1). These proteins act as competitive inhibitor for the physiological downstream targets of the particular GTPases in the cell. Instead of overexpression of plasmids coding for these proteins by transfection of the vessel preparation, the authors constructed and purified fusion proteins of the inhibitors and the HIV-TAT sequence. Proteins carrying the TAT motif are rapidly taken up by cells and this approach has been used to reliably transfer peptides and even proteins into cells in culture and also in vivo. Shiga et al are well aware of the fact that such an approach might be associated with unspecific effects and thus provide several control experiments validating the technique in their model. With the use of this elegant protein transfer technique, the authors provide evidence that only RhoA, but not Rac-1 or Cdc42 controls eNOS expression. As a physiological readout, contractile responses of rabbit mesenteric arteries were studied and were indeed observed to be attenuated by the RhoA-inhibitory protein via a mechanism involving NO.

The effect of the RhoA-inhibitory protein was put in relation to those of the HMG-CoA-reductase inhibitor simvastatin. As expected, simvastatin also increased eNOS expression and attenuated contractile responses. Interestingly, the inhibitory effect of simvastatin on contraction was only partially dependent on NO and this was restricted to a single concentration of simvastatin (30 μmol/L). Lower concentrations had no effect whereas 100 μmol/L simvastatin attenuated contraction in an endothelium-independent manner. Inhibition of RhoA using the TAT-coupled Rho-kinase binding domain had no effect following inhibition of eNOS, and the effect of simvastatin could only be partially antagonized by geranylgeranylprophosphate. The latter approach should restore RhoA isoprenylation. Therefore, the authors conclude that 100 μmol/L of simvastatin elicits “unspecific” effects. Such a conclusion might however not be justified. Different to monolayer cell culture models, the mesenteric artery of the rabbit is a complex tissue in which the local bioavailability may differ enormously for substances and for cell types. In fact, it appears likely that neither the large recombinant proteins nor the phospholipids used in this study may sufficiently penetrate the smooth muscle layer in the relatively thick-walled rabbit mesenteric artery. Given the central role of RhoA in the control of smooth muscle contraction, it would even be expected that inhibition of this GTPase attenuates contractile responses in an endothelium-independent manner.

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Without a doubt, NO elicits a plethora of beneficial effects in the organism. The positive effects of RhoA inhibition however will extend beyond the increase in NO bioavailability. Besides the above-mentioned effects on contraction and myogenic tone in smooth muscle cells, RhoA for example controls the contractile state of endothelial cells which determines permeability.9 More importantly, RhoA inactivates the insulin-receptor substrate-1 via Rho-kinase mediated phosphorylation10 and statins have been shown to activate several elements of the signal transduction cascade of the insulin receptor, including the protein kinase B/Akt.11 This observation may serve to explain the clinical observation that statins appear to improve insulin-sensitivity in type II diabetes.11

It is clear that an effect of RhoA inhibition requires active RhoA to be present in the cell. RhoA activity, however, is controlled by numerous pathways involving phosphorylation of RhoA,12 the activity and expression of guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), guanine-dissociation inhibitors (GDI),13 the activity of the geranyl-geranyl-transferase (GTF)14 and finally the supply with GTP and geranylgeranyl pyrophosphate. Consequently, there is little doubt that cardiovascular disorders are associated with RhoA activation, but the individual mechanism eliciting RhoA-activation remains obscure.

There is also uncertainty about the mechanism of action of RhoA on eNOS expression. It has been demonstrated that the effects of RhoA on contraction depend on Rho-kinases, which inactivate the myosin light chain phosphatase.8 Consequently, inhibition of Rho-kinase using the pharmacological inhibitors Y-27632 or fasudil has been shown to lower blood pressure in animal models of hypertension15 and to reduce vascular resistance in hypertensive individuals.16 Shiga et al reported that at least in the rabbit mesenteric artery a direct inhibition of Rho-kinase using Y-27632, in contrast to statins and RhoA-inhibitory proteins, had no effect on eNOS protein expression.6 This may suggest that effectors of RhoA, different from Rho-kinase mediate the effect observed. It has been demonstrated that eNOS mRNA interacts with nonfilamentous actin (G-actin).19 Actin polymerization, indeed, is mediated by RhoA in a Rho-kinase independent manner involving the mammalian homologue of diaphanous (mDia)3 and this mechanism seems to be responsible for the effects observed in the present study (Figure 2). Nevertheless, it should be noted that there is also evidence that Rho-kinase plays a role in the control of eNOS expression: Thrombin and hypoxia downregulate eNOS in a Rho-kinase inhibitor sensitive way.17,18 Indeed, Rho-kinase also has links to the actin organization through LIM kinase and the ezrin-radixin-moesin (ERM) family of proteins but the precise mechanisms underlying Rho-kinase mediated inhibition of eNOS expression still needs to be determined.

In conclusion, inhibition of RhoA is an attractive pharmacological approach for the prevention of cardiovascular disorders. Upregulation of eNOS is only one of the positive effects arising from inhibition of RhoA. Despite the strong...
rational arising from studies like the one published by Shiga et al., clinical trials will teach whether inhibition of RhoA will yield the anticipated benefits in patients.

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References

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