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Rapid communication

Long term Rho-kinase inhibition ameliorates endothelial dysfunction in LDL-Receptor deficient mice

Kerstin Steioff, Hartmut Rütten, Andreas E. Busch, Oliver Plettenburg, Yuri Ivashchenko, Matthias Löhn*

Sanofi-Aventis Pharma GmbH, Therapeutic Department Cardiovascular Diseases, Industriepark Höchst, 65926 Frankfurt am Main, Germany

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Abstract

Chronic inhibition of Rho-kinase has been recently implicated in retardation of atherogenesis induced by high-fat diet in low-density lipoprotein receptor deficient ($LDLR^{-/-}$) mice. However, it remains to be examined whether long-term Rho-kinase inhibition will reduce vascular dysfunction in this model. $LDLR^{-/-}$ mice on a high-fat diet were treated either with saline ($LDLR^{-/-}$) or with the Rho-kinase inhibitor Fasudil (HA1077, 5-Isoquinolinesulfonyl homopiperazine, 100 mg/kg/day by gavage, $LDLR^{-/-}$+Fasudil) for 10 weeks. Fasudil-treatment normalized endothelial function (measured by means of endothelium-dependent vasorelaxation) in $LDLR^{-/-}$+Fasudil, to the level of controls (C57BL/6J). No tolerance toward Rho-kinase inhibition has been detected in Fasudil-treated animals. We conclude that long-term Rho-kinase inhibition normalizes endothelial function without development of tolerance.

Keywords: Hypercholesterolemia; Rho-kinase; Endothelial dysfunction

Low-density lipoprotein receptor deficient ($LDLR^{-/-}$) mice are characterized by hypercholesterolemia upon dietary cholesterols (Ishibashi et al., 1993). Recently, it has been demonstrated that long-term Rho-kinase inhibition in $LDLR^{-/-}$ mice showed anti-atherogenic and anti-inflammatory effects (Mallat et al., 2003). This raised the question whether long-term Rho-kinase inhibition could also improve endothelial dysfunction present in $LDLR^{-/-}$ mice (Rabelo et al., 2003). Adult male C57BL/6J and $LDLR^{-/-}$ mice (9–11 weeks old, 25–35 g, Jackson Laboratory, Maine, USA) were used in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). Controls, C57BL/6J ($n=10$) received conventional chow, $LDLR^{-/-}$ ($n=10$) were placed on a high-fat diet (15% cacao butter, 1.25% cholesterol, Charles River, Germany), and $LDLR^{-/-}$+Fasudil ($n=10$) received a high-fat diet and were orally treated with HA1077 (Fasudil, 5-Isoquinolinesulfonyl homopiperazine, a specific Rho-kinase inhibitor (Davies et al., 2000) at 100 mg/kg/day for 10 weeks) with free access to food and water. Vascular function was assessed by standard organ bath techniques. Mice were sacrificed by decapitation, the thoracic aortas were excised and then quickly transferred to cold (4 °C) oxygenated with carbogen (95% O_2 and 5% CO_2) physiological salt solution. Aortas were cleaned and dissected into 5-mm rings as described previously (Lohn et al., 2005). The composition of the bath solution (in mM) was 119 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 25 $NaHCO_3$, 1.2 $MgSO_4$, 11 glucose, 1.6 $CaCl_2$. Bath solutions were continuously gassed with carbogen to provide oxygenation and pH of 7.4. The temperature was maintained at 37 °C. Aortas were precontracted by KCl (60 mM), and after washout, by phenylephrine (5×10^{-7} M) and cumulative concentration–response curves were obtained for acetylcholine (1, 5×10^{-9}, 10^{-8}, 2×10^{-8}, 1, 2, 5×10^{-7} and 1, 2×10^{-6} M). From each animal, three aortic rings were used. Data

* Corresponding author. Tel.: +49 69 305 17118; fax: +49 69 305 29092.
E-mail address: matthias.loehn@sanofi-aventis.com (M. Löhn).

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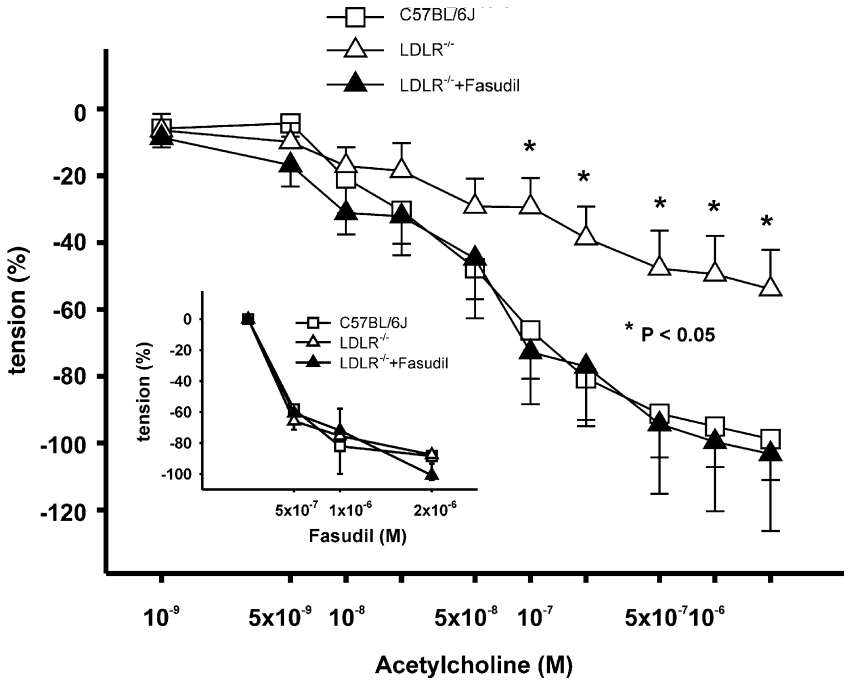

Fig. 1. Endothelium-dependent relaxation in mouse aortas. In contrast to aortas of control C57BL/6J, endothelium-dependent relaxations are significantly reduced in aortas of LDLR$^{-/-}$. Long-term Rho-kinase inhibition normalizes endothelial function in aortas of LDLR$^{-/-}$+Fasudil. Insert: Test for tolerance development after long-term Rho-kinase inhibition in vivo. Aortas of all groups were relaxed by Fasudil in a similar concentration-dependent manner.

are expressed as mean ± S.E.M. Differences between groups were tested for significance using one-way repeated measures analysis of variance. All statistical analysis were considered significant when $P<0.05$. The contractile responses to KCl were similar in all groups. In aortas of control group, acetylcholine induced a dose-dependent relaxation with an average $ED_{50} < 10^{-7}$ M. In contrast, LDLR$^{-/-}$ showed a significantly reduced relaxation elicited by acetylcholine with an $ED_{50} \sim 10^{-6}$ M, indicating that endothelial function is compromised (Fig. 1). Acetylcholine (2×10^{-6} M) induced complete relaxation in aortas of C57BL/6J (99 ± 12%) and LDLR$^{-/-}$+Fasudil (100 ± 23%) mice when in aortas of LDLR$^{-/-}$ mice only 54 ± 12% relaxation has been achieved with this concentration of acetylcholine. This indicate that compromised endothelial function can be normalized by long-term Rho-kinase inhibition. In order to determine whether long-term treatment of animals with Rho-kinase inhibitors will lead to tolerance toward Rho-kinase inhibition, we have tested the relaxation responses to Fasudil. Aortic rings of C57BL/6J, LDLR$^{-/-}$, and LDLR$^{-/-}$+Fasudil (at the end of the 10th week) were precontracted with phenylephrine (5×10^{-7} M) and thereafter, concentration-dependent relaxation responses of aortas to Fasudil (5×10^{-7}, 1 and 2×10^{-6} M) were compared. Aortas of all groups relaxed upon Rho-kinase inhibition in a similar manner with $ED_{50} < 5\times10^{-7}$ M Fasudil (Fig. 1, insert). We conclude that long-term treatment of animals with a Rho-kinase inhibitor does not result in development of tolerance toward acute application of the inhibitor. The present study demonstrates that long-term inhibition of Rho-kinase normalizes endothelial dysfunction in LDLR$^{-/-}$ mice (Mallat et al., 2003). Recently, hypercholesterolemia has been linked to the decreased bioavailability of nitric oxide as a result of stress fiber formation via Rho-kinase activation and subsequent translocation of endothelial nitric oxide synthase to caveolae with concomitant inactivation of the enzyme (Zhu et al., 2003). Therefore, it is possible that inhibition of Rho-kinase prevents inactivation of endothelial nitric oxide synthase, thereby elevating the bioavailability of vasodilating nitric oxide. Importantly, we have found no signs of developing tolerance development toward Rho-kinase inhibition after long-term application of Fasudil in vivo. Therefore, Rho-kinase inhibition may represent a new efficient therapeutical strategy to normalize endothelial dysfunction in hypercolesterolemic patients.

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