Endothelial nitric oxide synthase is essential for the HMG-CoA reductase inhibitor cerivastatin to promote collateral growth in response to ischemia[1]

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SPECIFIC AIMS

We addressed the hypothesis that HMG-CoA reductase inhibitors, or statins, can augment collateral flow to ischemic tissues by activating endogenous endothelial nitric oxide synthase (eNOS). By studying the effects of cerivastatin on blood-flow recovery after acute hindlimb ischemia in eNOS+/+ and eNOS−/− mice, we demonstrate the essential role of eNOS for cerivastatin to promote collateral growth in ischemic tissues.

PRINCIPAL FINDINGS

1. Cerivastatin enhances blood-flow recovery after acute ischemia in wild-type mice

To evaluate the angiogenic effect of cerivastatin, we generated hindlimb ischemia in C3H/He mice and C57BL/6J mice, which were treated with saline or cerivastatin. The blood flow of the ischemic and nonischemic legs was monitored weekly by laser Doppler imaging (**Fig. 1*A***). In the control C3H/He mice, the blood flow of the ischemic leg recovered gradually, reaching half the blood flow of the untreated leg by wk 5 (Fig. 1*B*). Cerivastatin dramatically enhanced the blood-flow recovery. In the mice treated with cerivastatin, blood flow in the ischemic leg recovered to almost that of the untreated limb in 2 wk. Cerivastatin also enhanced blood-flow recovery after acute ischemia in C57BL/6J mice (Fig.1*C*).

Autoamputation of the ischemic toe was frequently (five of six) observed in the control C3H/He mice, whereas no amputation was observed in the mice treated with cerivastatin. Collateral formation was evaluated by the capillary density of the ischemic hindlimb muscle harvested 5 wk after surgery. Consistent with the measurement by laser Doppler imaging, anti-CD31 immunostaining revealed that cerivastatin significantly increased the number of detectable capillaries in the ischemic leg.

2. Cerivastatin enhances eNOS activity and promotes vasodilatation of capillaries

Accumulating evidence indicates that nitric oxide (NO) production by endothelial cells mediates the angiogenic effect of many growth factors. We studied the effect of cerivastatin on eNOS activity. The endothelium-dependent relaxation of precontracted aortic rings was markedly enhanced in the aorta from mice treated with cerivastatin, demonstrating the enhancement of eNOS activity by cerivastatin.

3. Cerivastatin fails to promote blood-flow recovery in eNOS-deficient mice

To further investigate the critical role of eNOS in enhancing blood-flow recovery by cerivastatin, we evaluated the effects of cerivastatin in eNOS-deficient mice. These mice lack the ability to dilate the capillary via NO production in the endothelial cells. Surgical resection of the right femoral artery induced hindlimb ischemia in eNOS−/− mice similar to that of wild-type mice. However, blood-flow recovery was severely impaired in eNOS −/− mice, as reported previously. In contrast to the dramatic enhancement of collateral development observed in wild-type mice, cerivastatin had no beneficial effects on blood-flow recovery in eNOS −/− mice (**Fig. 2*A***). Anti-CD31 immunostaining revealed that cerivastatin treatment did not induce an increase in the number of visible capillaries in the ischemic leg (Fig. 2*B, C*).

CONCLUSIONS

In this study, we demonstrate that cerivastatin markedly enhanced blood-flow recovery after acute ischemia.

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.01–0415fje; to cite this article, use *FASEB J.* (September 17, 2001) 10.1096/fj.01–0415fje

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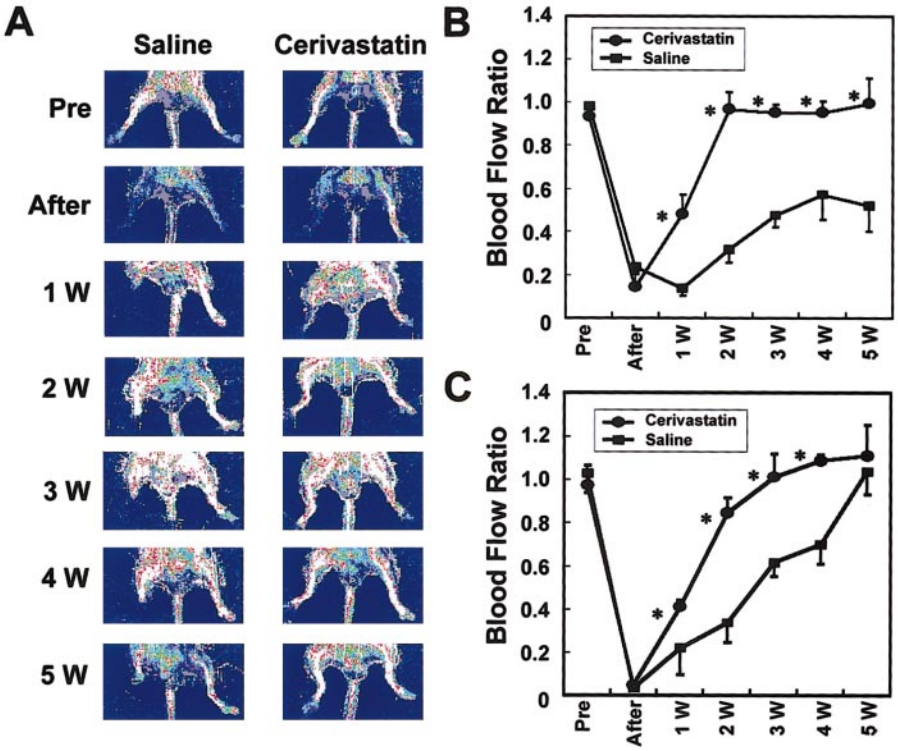

Figure 1. Promotion of blood-flow recovery by cerivastatin. Unilateral hindlimb ischemia was induced in 30- to 35-wk-old male wild-type C3H/He mice (*A, B*) and C57BL/6J (*C*) by resecting the right femoral artery. Hindlimb blood perfusion was measured with a laser Doppler perfusion imager system (*A*). Either saline or cerivastatin (6 mg/kg/day) was administered subcutaneously every day starting 3 days before surgery. The blood flow of the ischemic hindlimb was expressed as the ratio to that of the uninjured limb (*B, C*).

Blood-flow recovery correlated with the increase in the number of detectable capillaries. Endothelial NOS activity was enhanced in the mice treated with cerivastatin. Cerivastatin failed to promote blood-flow recovery when eNOS was genetically ablated. These results provide genetic evidence that eNOS is essential for cerivastatin to promote collateral growth in response to tissue ischemia in mice. Our findings suggest that statins may have a new potential for therapeutic angiogenesis.

When this study was being conducted recently, it was reported that the HMG-CoA reductase inhibitor simvastatin promoted ischemia-induced angiogenesis in normocholesterolemic rabbits. The authors demonstrated that statins activated the protein kinase Akt in vascular endothelial cells and that gene delivery of the constitutive active form of Akt to the endothelium mimicked the angiogenic effect of VEGF or simvastatin. The authors concluded that activation of Akt represented a mechanism that could account for some of the beneficial side effects of statins, including the promotion of new blood vessel growth. Activation of Akt plays a central role in various models of angiogenesis and Akt has been shown to mediate many biological effects induced by angiogenic growth factors, including VEGF and fibroblast growth factor, which promote all kinds of angiogenesis nonselectively. In contrast to these angiogenic growth factors, there is no clinical evidence that

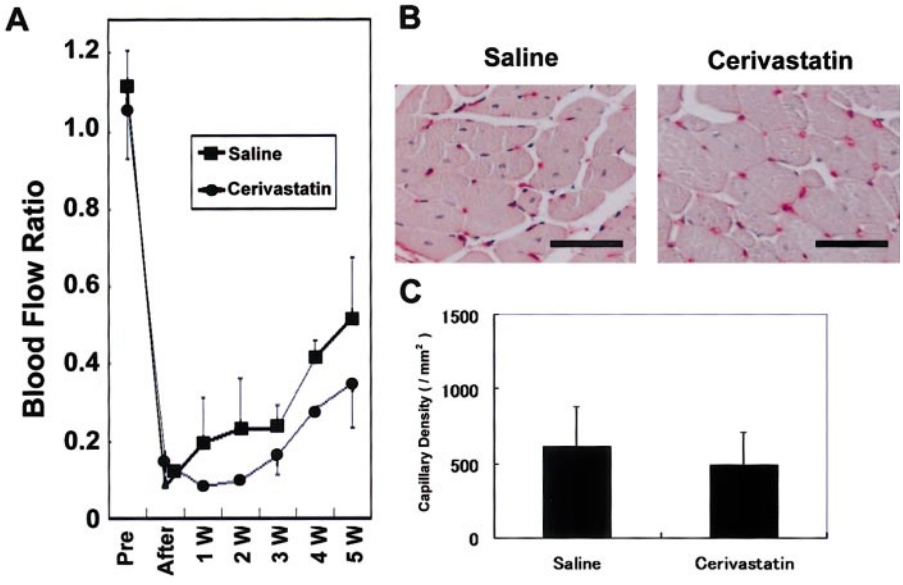

Figure 2. Failure of cerivastatin to improve blood-flow recovery in eNOS-deficient mice. *A, B)* Hindlimb ischemia was induced in 30- to 35-wk-old male eNOS −/− mice. The mice were treated with either saline or cerivastatin (6 mg/kg/day) (n=5 for each group). Blood-flow recovery was monitored weekly by a laser Doppler imager. *C)* The ischemic muscles were harvested from eNOS−/− mice 5 wk after surgery. Capillary density was measured by anti-CD31 immunostaining.

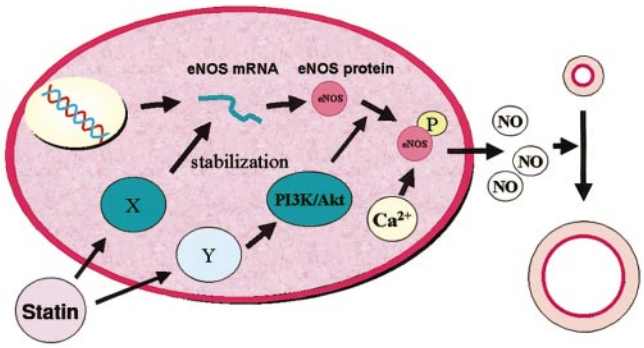

Figure 3. Schematic diagram of hypothesized eNOS involvement in the angiogenic effects of statins. Statins up-regulate eNOS expression through stabilization of its mRNA. In addition, statins activate protein kinase Akt, which enhances eNOS activity leading to NO production. Thus, statins seem to augment eNOS activity via two pathways: up-regulation of protein expression and the phosphorylation-dependent activation of its function. NO promotes vasodilatation of capillaries, resulting in an increase in blood flow in ischemic tissues.

statin therapy promotes unfavorable angiogenesis associated with tumor growth, diabetic retinopathy, or atherosclerotic plaque destabilization. Conversely, statins have been suggested to suppress pathological angiogenesis, such as neovascularization in atheroma and tumor. Although the report by Kureishi et al. provided convincing evidence that activation of endothelial Akt mediates the angiogenic effect of simvastatin, the in vitro data they presented may not fully explain the differential effects of statins on beneficial and unfavorable angiogenesis. Although the work by Kureishi et al. has raised clinical enthusiasm that statins may be useful for therapeutic angiogenesis in patients with ischemic diseases, we need to further clarify the molecular mechanism by which statins differentially promote collateral vessel growth in ischemic tissues without inducing harmful angiogenesis before starting clinical evaluation of statins with regard to therapeutic angiogenesis.

Here, we provided mouse genetic evidence that eNOS mediates the angiogenic effect of statins. Statins have been shown to up-regulate eNOS expression at the protein level through stabilization of its mRNA. In addition, it was demonstrated that statins activate protein kinase Akt, which enhances eNOS activity leading to NO production. Thus, statins seem to augment eNOS activity via two pathways: up-regulation of protein expression and the phosphorylation-dependent activation of its function. Consistent with this notion is the observation by Kureishi et al. that statins can achieve potent angiogenic activity in vivo with relatively mild activation of Akt in vitro.

It remains to be elucidated how the activation of eNOS promotes ischemia-induced collateral formation. NO prolongs survival and promotes migration of endothelial cells but not their proliferation, unlike other angiogenic growth factors. Recent evidence indicates that blood flow can increase not only through 'angiogenesis', the sprouting of endothelial cells leading to capillary networks, but also by 'arteriogenesis', the growth of preexistent collateral arterioles into functional collateral arteries. The endothelium-dependent vasodilatation induced via eNOS activation appears to play an initial role in the process of arteriogenesis. Thus, it is plausible that activation of eNOS by statins favors the process of arteriogenesis in response to tissue ischemia. Consistent with this notion is our observation that cerivastatin dramatically increased the blood flow in ischemic muscle with a moderate increase in capillary density (Fig. 1*B* and Fig. 2*C*).

It has recently been reported that statin therapy increases circulating endothelial progenitor cells (EPCs) in patients with stable coronary artery diseases. Mobilization of EPCs and functional enhancement of their activity may contribute to the angiogenic effects by cerivastatin in addition to other effects of statins on endothelial cell survival, migration, or differentiation.

In conclusion, our findings elucidated the potential mechanism of a previously unappreciated action of statins, i.e., the enhancement of nutrient perfusion in ischemic tissues without inducing harmful angiogenesis. Pharmacological modification of eNOS activity by statins may hold the ideal rationale for therapeutic angiogenesis. Our findings may also lead to the identification of novel angiogenic substances on the basis of their activation of eNOS. FJ