Dr. Danias points out similar concerns to Drs. Shepard and Eisenberg regarding the low sensitivity and specificity for technetium-stress testing. As discussed previously, this is clearly a limitation of our study and could potentially be resolved by evaluating a larger number of patients. Dr. Danias also expresses concern over the 27 patients who had a positive EBCT scan and a negative treadmill-ECG and were therefore classified as having a negative "test" for the combined approach (EBCT combined with treadmill-ECG). The mean coronary calcium (CC) score for these patients as determined by the Agatston method (3) was 394, range 1 to 1420. For this combined approach, a positive EBCT was defined as a CC score >0 in order to maximize sensitivity. Raising the CC score cutoff would lower sensitivity and raise specificity, as shown in Table 3 of our article (1). We agree with Dr. Danias that our study did not include a cost-effectiveness analysis, which would be useful in further determining the utility of EBCT in the evaluation of symptomatic patients. However, EBCT does have a relatively low cost, and other studies have documented its benefit in the diagnostic evaluation of patients with symptoms suggestive of CAD (4,5).

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REFERENCES

1. Shavelle DM, Budoff MJ, LaMont DH, Shavelle RM, Kennedy JM, Brundage BH. Exercise testing and electron beam computed tomography in the evaluation of coronary artery disease. J Am Coll Cardiol 2000;36:32–8.
2. Fleischmann KE, Hunink MG, Kuntz KM, Douglas PS. Exercise echocardiography or exercise SPECT imaging? A meta-analysis of diagnostic test performance. JAMA 1998;280:913–20.
3. Agatston A, Janowitz W, Hildner F, Zusmer N, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15:827–32.
4. Kajinami K, Seki H, Takekoshi N, Mabuchi H. Noninvasive prediction of coronary artherosclerosis by quantification of coronary artery calcification using electron beam computed tomography: comparison with electrocardiographic and thallium exercise stress test results. J Am Coll Cardiol 1995;26:1209–21.
5. LaMont DH, Budoff MJ, Shavelle DM, Brundage BH, Hager JM. Coronary calcium scanning identifies patients with false positive stress tests (abstr). Circulation 1997;96:306–I.

Molecular Effects of HMG-CoA Reductase Inhibitors on Smooth Muscle Cell Proliferation

We read with great interest the report by Indolfi et al. (1). The data reported are very interesting because, to the best of our knowledge, this is the first report demonstrating simultaneously that: 1) a hydroxymethylglutaryl Coenzyme A (HMG-CoA) reductase inhibitor blocks smooth muscle cell (SMC) proliferation in vitro; 2) this inhibitor potently reduces neointimal formation induced by vascular injury in vivo; and 3) the in vitro and in vivo effects are completely abolished by mevalonate but not by cholesterol. The investigators linked the antiproliferative effect of the HMG-CoA reductase inhibitor to suppression of Ras farnesylation and the Ras-mediated MAPK (mitogen-activated protein kinase) transduction pathway.

However, we have evidence that the HMG-CoA reductase inhibitors have several targets (not only the Ras farnesylation) in the SMC proliferation, which have not been completely identified yet. This is in agreement with data of Grandaliano et al. (2), who have described that the inhibition of cell proliferation by simvastatin was not reversed by farnesol. Furthermore, Wejde et al. (3) have demonstrated that farnesol failed to promote the growth of compactin (a lovastatin analogue)-blocked cultured breast cancer cells. In addition, our data have shown that despite lovastatin-mediated inhibition of Ras farnesylation, the activation of MAPK is only partially inhibited (4).

Several lines of evidence suggest that the endogenous basic fibroblast growth factor (bFGF), known to be synthesized by vascular SMC (5,6), plays an important role in the stimulation of SMC proliferation that occurs during atherogenesis (7) and in response to vessel wall injury (8). Furthermore, it has been shown that i) bFGF, released from arterial SMC after injury, is a potent mitogen (9) and ii) bFGF- or injury-induced SMC proliferation is significantly inhibited by anti-bFGF antibodies (10). Thus, bFGF expressed by vascular SMC is a strong mitogenic factor stimulating SMC in an autocrine and paracrine manner. However, no studies about the association between the content of the endogenous bFGF and the HMG-CoA reductase inhibitor treatment of SMC were reported.

Thus, we have analyzed the effects of lovastatin on growth factor-induced DNA synthesis in a dose-dependent manner in human coronary SMC in vitro as well as the influence of the HMG-CoA reductase inhibitor on the expression of the endogenous bFGF. Our [^{3}H] thymidine and cell-counting experiments showed that lovastatin caused a reduction of the DNA synthesis and proliferation in human SMC in a dose-dependent manner. Mevalonate (50 μmol/liter) reduced the inhibition produced by lovastatin (5 μmol/liter) by 90%. In contrast, addition of cholesterol did not overcome the inhibition, demonstrating that these effects are not cholesterol-dependent. Furthermore, lovastatin treatment of SMC (in the concentration range that inhibited SMC proliferation) significantly ($p < 0.05$) reduced the level of the endogenous bFGF to 55% of control cells. The lovastatin-induced effects were reversed by mevalonate but not by cholesterol.

These findings suggest that HMG-CoA reductase inhibitors suppress cell proliferation by downregulation of the expression of the endogenous bFGF. In light of the present findings of Indolfi et al. (1) and our group, it is likely that HMG-CoA reductase inhibitors target several points in the mitogenic pathway of SMC. First, as described by Indolfi et al. (1), HMG-CoA reductase inhibitors block the farnesylation of Ras and the Ras- mediated activation of MAPK. Second, the inhibitors suppress the endogenous expression of the strong mitogen bFGF. Overall, we agree with the investigators that the growth-inhibitory effects of HMG-CoA reductase inhibitors are cholesterol-independent. The underlying mechanisms, however, still remain to be elucidated in further studies.

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REFERENCES

1. Indolfi C, Cioppa A, Stabile E, et al. Effects of hydroxymethylglutaryl Coenzyme A reductase inhibitor simvastatin on smooth muscle cell proliferation in vitro and neointimal formation in vivo after vascular injury. J Am Coll Cardiol 2000;35:214–21.
2. Grandaliano G, Biswas P, Choudhury GG, Abboud HE. Simvastatin inhibits PDGF-induced DNA synthesis in human glomerular mesangial cells. Kidney Int 1993;44:503–8.
3. Wejde J, Carlberg M, Hjertman M, Larsson O. Isoprenoid regulation of cell growth: identification of mevalonate-labelled compounds inducing DNA synthesis in human breast cancer cells depleted of serum and mevalonate. J Cell Physiol 1993;155:539–48.
4. Skaletz-Rorowski A, Müller JG, Eschert H, Waltenberger J, Breithardt G. The effect of lovastatin on bFGF-induced MAPK signaling in coronary smooth muscle cells via phosphatase inhibition. Eur Heart J 2000; Suppl. In Press.
5. Schmidt A, Skaletz-Rorowski A, Breithardt G, Buddecke E. Growth status-dependent changes of bFGF compartmentalization and heparin sulfate structure in arterial smooth muscle cells. Eur J Cell Biol 1995;67:130–4.
6. Skaletz-Rorowski A, Schmidt A, Breithardt G, Buddecke E. Heparin-induced overexpression of basic fibroblast growth factor, basic fibroblast growth factor receptor, and cell-associated proteoheparan sulfate in cultured coronary smooth muscle cells. Arterioscler Thromb Vasc Biol 1996;16:1063–9.
7. Raines EW, Ross R. Smooth muscle cells and the pathogenesis of the lesions of atherosclerosis. Br Heart J 1993;69:S30–7.
8. Ferns GAA, Stewart-Lee AL, Anggard EE. Arterial response to mechanical injury: balloon catheter de-endothelialization. Atherosclerosis 1992;92:89–104.
9. Klagsbrun M, Edelman ER. Biological and biochemical properties of fibroblast growth factors: implications for the pathogenesis of atherosclerosis. Arteriosclerosis 1989;9:269–78.
10. Lindner V, Reidy MA. Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against basic fibroblast growth factor. Proc Natl Acad Sci USA 1991;88:3739–43.

REPLY

Skaletz-Rorowski raises the issue that HMG-CoA reductase inhibitors have several targets in smooth muscle cell (SMC) proliferation that have not been completely identified yet. We agree with Skaletz-Rorowski and associates that further studies should be performed in order to understand the molecular mechanisms responsible for the antiproliferative effects of HMG-CoA reductase inhibitors, and we have focused the future research of our laboratory on this important issue. However, the aims of our study were to assess the effects of the HMG-CoA reductase inhibitor simvastatin 1) on smooth muscle cell growth in vitro and 2) on neointimal formation after balloon angioplasty or arterial stenting (1). We demonstrated for the first time, in a model of arterial injury, that the HMG-CoA reductase inhibitor simvastatin reduced the neointimal hyperplasia in vivo and that this effect was abolished using local administration of mevalonate (1). These data might stimulate further studies to evaluate the effects of HMG-CoA reductase inhibitors in a stenting model of larger animals and eventually in humans.

A previous study from our laboratory demonstrated that the inhibition of cellular *Ras* using a transdominant negative *Ras* gene reduced significantly the neointimal formation after balloon injury (2). It is also well known that the HMG-CoA reductase inhibitors not only reduce plasma cholesterol levels but also competitively inhibit intracellular synthesis of mevalonate, a precursor of nonsterol compounds such as geranyl-geranyl and farnesyl. This effect on the synthesis of farnesyl radicals inhibits the *Ras* pathway, a key signal transducer that couples the receptors for diverse extracellular signals to different effectors (3).

Skaletz-Rorowski pointed out that bFGF plays an important role on SMC proliferation and that HMG-CoA reductase inhibitors may reduce the expression of this particular growth factor. Growth factors bind specific plasma membrane receptors and activate a complex network of intracellular kinase cascades.

However, it should also be pointed out that the activation of different membrane receptors of growth factors (including bFGF, IGF, EGF, VEGF, PDGF, PIGF, etc.) may induce SMC growth and are involved in the neointimal hyperplasia after vascular injury. In this redundant system, it is unlikely that the inhibition of a single growth factor will reduce the rate of clinical restenosis. Therefore, we believe that much interest should be focused on the intracellular common pathways (as the RAS-MAPKK [2] or cAMP-PKA [4]), key signal transducers that couple the receptors for diverse extracellular signals to different effectors. In this regard, the HMG-CoA reductase inhibitors are good clinical candidates to inhibit common pathways of intracellular kinase cascades.

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REFERENCES

1. Indolfi C, Cioppa A, Stabile E, et al. Effects of hydroxymethylglutaryl Coenzyme A reductase inhibitor simvastatin on smooth muscle cell proliferation in vitro and neointimal formation in vivo after vascular injury. J Am Coll Cardiol 2000;35:214–21.
2. Indolfi C, Avvedimento EV, Rapacciuolo A, et al. Inhibition of cellular RAS prevents smooth muscle cell proliferation after vascular injury in vivo. Nat Med 1995;6:541–5.
3. Indolfi C, Chiariello M, Avvedimento EV. Selective gene therapy of proliferative disorders: sense and antisense. Nat Med 1996;6:634–5.
4. Indolfi C, Avvedimento EV, Di Lorenzo E, et al. Activation of cAMP-PKA signalling in vivo inhibits smooth muscle cell proliferation induced by vascular injury. Nat Med 1997;3:775–9.