Direct inhibitory effects of simvastatin on matrix accumulation in cultured murine mesangial cells

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Background. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors have been demonstrated to suppress glomerular injuries in various renal diseases. These agents inhibit in vitro proliferation of several cell types, including mesangial cells. This effect indicates the ability to ameliorate mesangio-proliferative lesions, independent of the improvement of hypercholesterolemia. On the other hand, it is not clear whether HMG-CoA reductase inhibitors directly regulate extracellular matrix (ECM) accumulation from mesangial cells.

Methods. In this study, to examine the in vitro effects of simvastatin, an HMG-CoA reductase inhibitor, on mRNA expressions of matrix proteins, growth factors, and matrix turnover proteins, we incubated cultured murine mesangial cells stimulated by fetal calf serum (FCS) with or without simvastatin for 24 hours, and Northern analysis was performed.

Results. Simvastatin showed a slightly suppressive effect on mRNA expression of type IV collagen and fibronectin and a slightly up-regulative effect on that of type I collagen, whereas mRNA expression of type III collagen was markedly up-regulated. mRNA expression of platelet-derived growth factor (PDGF)-B chain and PDGF receptor β-subunit was suppressed, whereas that of transforming growth factor-β (TGF-β) was not affected by simvastatin. Concerning matrix turnover proteins, simvastatin markedly reduced mRNA expression of plasminogen activator inhibitor-1 (PAI-1) without affecting the expression of tissue-type plasminogen activator (tPA).

Conclusion. These results suggest type-specific modulation of matrix protein production independent of TGF-β and the suppressive effects of autocrine PDGF by administration of HMG-CoA reductase inhibitors in mesangial cells. Moreover, the beneficial effects of these agents on matrix protein accumulation may be through promoting ECM degradation derived from PAI-1 suppression.

METHODS

Murine mesangial cell culture

Twenty-four-week-old BALB/c mice were sacrificed, and the kidneys were removed in a sterile manner. Glomerular mesangial cells were cultured according to the methods of MacKay et al [6]. Cells obtained from passages 5 to 12 were used for the experiments.
Simvastatin and mevalonate preparation

Simvastatin salt was kindly provided by Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan) and was prepared as described by Kita et al [7]. Mevalonic acid lactone was prepared as described by O’Donnell et al [8]. Aliquots of these solutions were stored at -20°C until use.

RNA purification and Northern analysis

Preconfluent mesangial cells were cultured in fibronectin-coated 100 mm dishes for 48 hours in serum-free modified Eagle’s medium (MEM)/F12 medium (GIBCO BRL, Gaithersburg, MD, USA) to render the cells quiescent and were then further cultured for 24 hours in MEM/F12 medium with the following additions: 10% fetal calf serum (FCS) only, as a control; 10% FCS and 20 μM of simvastatin; and 10% FCS, 20 μM of simvastatin, and 100 μM of mevalonate. Total RNA of cultured mesangial cells was purified by the acid guanidium thiocyanate-phenol-chloroform method, and Northern blot analysis was performed as previously described [9]. Each lane of the autoradiographs was analyzed with BAS-2000 (Fuji film, Tokyo, Japan), and the signal densities of each mRNA, corrected using that of GAPDH, were compared among the conditions mentioned earlier here. The expression of the following mRNA was examined: matrix proteins such as collagen type I (α1), type III (α1), type IV (α1), and fibronectin; growth factors such as PDGF-B chain, PDGF-receptor β-subunit (PDGF-Rβ), and transforming growth factor-β (TGF-β); and matrix turnover proteins such as matrix metalloproteinase 2 (MMP-2), tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), and PAI-1.

RESULTS

Simvastatin slightly suppressed mRNA expression of collagen IV and fibronectin and slightly up-regulated that of collagen I; however, a marked increase in collagen III mRNA expression was noted. mRNA expression of PDGF-B chain and PDGF-Rβ was markedly suppressed in contrast to no influence on TGF-β mRNA expression. Among the matrix turnover proteins, although MMP-2 and tPA showed no significant changes in gene expression, uPA and PAI-1 mRNA expression was significantly reduced by simvastatin. The nearly complete elimination of these effects by the addition of mevalonate indicates the direct influence of simvastatin on these parameters (Fig. 1 and Table 1).

DISCUSSION

These results show type-specific regulation of matrix protein synthesis of mesangial cells by simvastatin. The distinction between the regulation of type III and type IV collagens was also demonstrated by cyclosporin A and interleukin-4 [10, 11]. Considering that the in vitro synthesis of type I and III collagens was enhanced in cultured mesangial cells and that of type IV collagen was up-regulated in vivo, the signaling pathway affected by simvastatin for these matrix protein syntheses may be completely different.

Grandaliano et al reported that under PDGF-induced conditions, simvastatin did not influence mRNA expression of PDGF-B chain, although simvastatin completely inhibited up-regulated DNA synthesis induced by PDGF stimulation [4]. In contrast, we detected marked suppression of PDGF expression in murine mesangial cells by
Simvastatin, along with complete suppression of DNA synthesis under FCS-stimulated conditions (data not shown), as seen by Grandaliano’s group. Considering the difference between these two studies in the conditions that induced up-regulated cell proliferation, simvastatin may inhibit some FCS-induced pathway(s) other than that of PDGF, which induces the expression for mRNA of PDGF-B and its receptor. This is supported by a report of human fibroblasts in which lovastatin has suppressed a signaling response to PDGF less intensely than to epidermal growth factor (EGF) or insulin-like growth factor I (IGF-I) [12]. In addition to the direct induction of cell cycle arrest by simvastatin, indirect intervention through the suppression of mRNA expression of PDGF-related proteins may be related to the inhibition of DNA synthesis in vitro. PDGF down-regulation in mesangial cells by simvastatin may exert beneficial effects on inflammatory glomerular lesions through the suppression not only of cell proliferation but also of the resultant matrix synthesis. On the other hand, the effects of simvastatin on matrix protein synthesis in cultured murine mesangial cells appear to be independent of TGF-β, a potent fibrinogenic peptide widely known to mediate mesangial ECM expansion and glomerular sclerosis in various renal diseases.

Simvastatin has no specific influence on MMP-2 mRNA expression in mesangial cells; however, concerning serine proteinase expression, our findings showed a relatively heterogeneous effect. Almost no effect on tPA but a markedly suppressive effect on uPA and PAI-1 mRNA expression was demonstrated in simvastatin-stimulated mesangial cells. Au, Kenagy, and Clowes reported that in vascular smooth muscle cells, heparin suppresses 4 β-phorbol-12-Myristate-13-acetate (PMA) or serum-induced tPA mRNA expression, whereas it has little effect on that of uPA and suggested that the induction of tPA but not uPA is dependent on the activity of protein kinase C (PKC) [13]. The remarkably differential action of simvastatin on the expression of PAs may reflect its lack of effect on PKC activity [4]. As it has been reported that the physiological role of tPA is predominant to that of uPA [14] and that tPA deficiency exacerbates experimental glomerulonephritis lesions to a greater extent than uPA deficiency [15], the absence of an effect on tPA expression indicates that simvastatin may not disturb serine proteinase activity at least in the glomerulus. In addition, the marked down-regulation of PAI-1 expression may contribute beneficial effects on glomerular lesions in spite of uPA down-regulation. This may be an additional mechanism by which HMG-CoA reductase inhibitors improve renal disease.

In summary, simvastatin modulates ECM production in a type-specific manner independent of TGF-β and inhibits cell growth in part through the suppressive effects of autocrine PDGF. Moreover, simvastatin suppresses PAI-1 expression with the steady expression of tPA in mesangial cells. Part of the beneficial effects of HMG-CoA reductase inhibitors in renal diseases may be through its effect on ECM by enhancing matrix protein degradation.

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