Statin Treatment of Adult Human Glial Progenitors Induces PPARγ-Mediated Oligodendrocytic Differentiation

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ABSTRACT
The statins have been proposed as possible therapeutic agents for a variety of autoimmune disorders, including multiple sclerosis. In a genomic screen, we found that glial progenitor cells (GPCs) of the adult human white matter expressed significant levels of the principal statin target, HMG-CoA reductase, as well as additional downstream members of the sterol synthesis pathway. We therefore asked if statin treatment might influence the differentiated fate of adult glial progenitor cells. To assess the functional importance of the sterol synthesis pathway to adult glial progenitors, we used simvastatin or pravastatin to inhibit HMG-CoA reductase, and then assessed the phenotypic differentiation of the progenitors, as well as the molecular concomitants thereof. We found that both statins induced a dose-dependent induction of oligodendrocyte phenotype, and concomitant reduction in progenitor cell numbers. On that basis, we investigated the effects of statins on adult glial progenitor cells. The statins induced a rapid and substantial, dose-dependent induction of oligodendrocyte phenotype, and then assessed the phenotypic differentiation of the progenitors, as well as the molecular concomitants thereof. We found that both statins induced a dose-dependent induction of oligodendrocyte phenotype, and concomitant reduction in progenitor cell number. Oligodendrocyte commitment was associated with induction of the sterol-regulated nuclear co-receptor PPARγ, and could be blocked by the specific PPARγ antagonist GW9662. Thus, statins may promote oligodendrocyte lineage commitment by parenchymal glial progenitor cells; this might reduce the available progenitor pool, and hence degrade the long-term regenerative competence of the adult white matter. ©2008 Wiley-Liss, Inc.

INTRODUCTION
The statin family of cholesterol-lowering drugs, such as simvastatin and pravastatin, act as HMG-CoA reductase inhibitors, and have been proposed as potential candidates for the treatment of multiple sclerosis (MS) (Bradbury, 2002; Stanislaus et al., 1999). A number of studies have suggested that statins may ameliorate the signs and symptoms of acute inflammatory demyelinating disease by modulating the neuroinflammatory response (Paintlia et al., 2004; Stanislaus et al., 1999; Vollmer et al., 2004; Youssef et al., 2002) (reviewed in Weber et al., 2005). In addition, complementary studies have focused on the effects of statins on myelin production and elaboration (Miron et al., 2007; Paintlia et al., 2005). Paradoxically, even though the major cholesterol producing cell-type in the CNS is the oligodendrocyte, statins have been found to potentiate both oligodendrocytic process elaboration and remyelination (Miron et al., 2007; Paintlia et al., 2005). To address the basis for the statin-associated potentiation of remyelination, we investigated the effects of statins on adult glial progenitor cells, which represent the principal, if not sole, source of new oligodendrocytes in the adult human CNS (Nunes et al., 2003; Roy et al., 1999). These glial progenitor cells are typically bipotential for astrocytes and oligodendrocytes in vivo, and mediate myelin-repair in MS (Franklin, 2002). In a genomics analysis of glial progenitor cells derived from the adult human white matter, we had previously identified a series of differentially expressed signaling pathways that characterize the adult GPC (Sim et al., 2006). Among these, we found that adult GPCs selectively over-expressed HMG-CoA reductase, as well as both sterol synthesis and sterol regulated pathway components. On this basis, we asked whether statin treatment and its attendant suppression of cholesterol synthesis might influence the phenotypic commitment or lineage restriction of adult glial progenitor cells.

To assess the functional importance of the sterol synthesis and signaling pathways to adult glial progenitors, we used the HMG-CoA reductase inhibitors simvastatin and pravastatin, prototypic hydrophobic and hydrophilic members of the statin family, respectively, to suppress HMG-CoA reductase activity in cultured adult human GPCs. The statins induced a rapid and substantial, dose-dependent induction of oligodendrocyte differentiation, and a concomitant reduction in progenitor cell numbers. On that basis, we investigated the effects of statins on the PPAR family of transcription factors, which have been reported to trigger differentiation in oligodendrocytes as well as other lipidogenic cells, and...
Statins are known to induce differentiation of glial progenitors, which is correlated with the induction of PPARγ. We found that statin-induced oligodendrocytic differentiation was accompanied by a concomitant depletion of competent progenitor cells.

**MATERIALS AND METHODS**

**Cell and Tissue Samples**

Adult human subcortical white matter was obtained from temporal lobe tissue removed from 16 patients at craniotomy, 13 for medication-refractory epilepsy, one for vascular malformation, and two for noncontiguous tumor resections (age 5–48 years; eight males and eight females). Samples were obtained from patients who consented to tissue use under protocols approved by the University of Rochester-Schroen Memorial Hospital Research Subjects Review Board. The tissues were prepared and white matter progenitor cells freshly isolated on the basis of A2B5-based immunomagnetic sorting, as previously described (Nunes et al., 2003).

**Statin Treatment and Drug Challenges**

GPCs were distributed onto 12-well plates coated with poly-L-ornithine and fibronectin at 5 × 10^4 cells/mL in DMEM/F12/N1 supplemented with 10 ng/mL bFGF (Sigma, St. Louis, MO), 10 ng/mL PDGF-AA (Sigma), and 2 ng/ml NT3 (R&D Systems). GPCs were challenged with simvastatin (Calbiochem, San Diego, CA) or pravastatin (Calbiochem) at dosages of 0, 5, 10, 30, and 100 μM. Drug exposures or vehicle were incubated immediately upon plating and sustained for 7 or 14 days in vitro (DIV). Control cultures were also treated with 100 μM of the statin antagonist mevalonate (Sigma) concurrently with either 30 μM of simvastatin or 100 μM of pravastatin. In another set of cultures, the PPARγ-selective and irreversible antagonist GW9662 (Sigma) was added at dosages of 0, 1, or 10 μM, with or without concurrent treatment with 100 μM pravastatin, for 7 DIV.

**Immunocytochemistry**

Cultures were exposed continuously to 10 μg/mL BrdU beginning 24 h before fixation. After 7 to 14 days in vitro, the cultures were immunostained for the early progenitor and oligodendrocyte markers, A2B5 and O4, respectively, as described (Roy et al., 1999; Sim et al., 2006; Windrem et al., 2004). Both O4 and A2B5 were localized on live cells that were then fixed with 4% paraformaldehyde and immunostained for BrdU. O4 supernatant (gift of R. Bansal and S. Pfeiffer, University of Connecticut) was used at a dilution of 1:100, and monoclonal antibody A2B5 supernatant (clone 105, American Type Culture Collection) was diluted in a 1:1 with DMEM/F12/N1, each was applied for 40 min at 4°C. Rat anti-BrdU antibody (Oxford Biotechnology, Kidlington, UK) was used at a dilution of 1:200. Secondary antibodies, FITC, Alexa-594 and 647 conjugated goat anti-mouse IgG, rabbit and rat antibodies, respectively, were used at a dilution of 1:400 (Invitrogen, La Jolla, CA). Fixed cultures were counterstained with DAPI (10 ng/mL; Invitrogen). The number of A2B5 and O4 stained and unstained cells were counted in 10 randomly chosen fields from individual replicate samples (n = 3 at each dosage level). Statistical significance was assessed by one-way repeated measures analysis of variance (ANOVA), followed by Bonferroni’s multiple comparisons test (GraphPad Prism 4.03, P < 0.05).

**Real-Time RT-PCR Analyses**

RNA was extracted using Trizol (Invitrogen) and purified using RNeasy (Qiagen, Chatsworth, CA) as previously described (Sim et al., 2006). Primers and probes were obtained as Assays-on-Demand from Applied Biosystems (see Table 1 for details). We used 900 nM forward and reverse primers, and 250 nM FAM-labeled MGB probes. Real-time PCR analysis was performed using specific primers as described in Table 1, and expression data normalized to 18S rRNA. The relative abundance of transcript expression was calculated by ΔΔCt analysis. For real-time PCR analyses following statin treatment, three separate reverse transcription reactions of 25 ng total RNA were performed as per manufacturer’s protocol, and the resulting cDNA diluted to 100 pg/μL. Three separate real-time PCR reactions with 500 pg/reaction, in addition two no-RT control reactions were performed to rule out RNA-independent product amplification.
RESULTS

Adult GPCs Expressed High Levels of HMGCoA Reductase While Repressing PPARγ mRNA

In a recent whole-genome assessment of differential gene expression by human glial progenitor cells, which had been isolated from adult white matter by A2B5-based sorting (Sim et al., 2006), we found that a number of genes involved in sterol biosynthesis and metabolism were enriched in these cells (see http://www.urmc.rochester.edu/goldmanlab/sim2006.htm). Prominent among these was 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the rate-limiting enzyme in cholesterol biosynthesis. On this basis, we asked in this study if cholesterol synthesis inhibition might influence progenitor cell turnover or fate. We first confirmed that HMGCR was over-expressed by isolated GPCs, by a factor of 2.4-fold relative to the white matter dissociates from which these cells derived (Table 1). We validated this observation by real-time quantitative PCR (qPCR) of HMGCR mRNA (ΔΔCt analysis, paired t-test, P < 0.05; Table 1). We also observed higher expression of the cholesterol homeostasis signaling component, INSIG1 (Li et al., 2003), as well as the low-density lipoprotein receptor (LDLR), which acts to increase the availability of intracellular cholesterol. On this basis, we asked in this study if cholesterol synthesis inhibition might influence progenitor cell turnover or fate. We first confirmed that HMGCR was over-expressed by isolated GPCs, by a factor of 2.4-fold relative to the white matter dissociates from which these cells derived (Table 1). We validated this observation by real-time quantitative PCR (qPCR) of HMGCR mRNA (ΔΔCt analysis, paired t-test, P < 0.05; Table 1). We also observed higher expression of the cholesterol homeostasis signaling component, INSIG1 (Li et al., 2003), as well as the low-density lipoprotein receptor (LDLR), which acts to increase the availability of intracellular cholesterol. The latter was expressed 3.2-fold higher in GPCs than in the white matter (paired t-test, false discovery rate (FDR) <10% for all comparisons). Of particular note, we observed the selective down-regulation of the sterol-regulated transcription factor PPARγ—whose expression is typically suppressed by cholesterol synthesis (Inoue et al., 2000)—by a factor of 5.9 ± 0.2 fold. Using qPCR, we confirmed that PPARγ mRNA was significantly depleted in WMPCs when compared with the unsorted parental white matter (Table 1). Since both PPARγ and PPARδ (Saluja et al., 2001) agonists have been associated with the oligodendrocytic differentiation of rat glial progenitors, these data suggested the potential importance of intracellular sterol signaling, and its pharmacological modulation, to the fate decisions of adult human glial progenitor cells.

Simvastatin and Pravastatin Induced GPC Oligodendrocytic Differentiation In Vitro

To test the importance of sterol signaling to the differentiated fate of adult human GPCs, we challenged isolated A2B5+ glial progenitor cells with either simvastatin or pravastatin, and assessed their proliferation, phenotypic differentiation, and survival. Simvastatin and pravastatin were used as prototypic hydrophobic and hydrophilic statins, respectively (Quion and Jones, 1994). When raised in conditions compatible with their mitotic expansion, in serum-free media supplemented with PDGF/FGF/NT3 (Nunes et al., 2003), GPCs exposed for 7 days to 30 μM simvastatin exhibited a dose-dependent increase in O4-imunoreactive oligodendrocytes, from 23.7 ± 3.5% to 46.3 ± 4.1% of all cells (n = 3 patient samples, each experiment in triplicate) (Fig. 1). O4 was
used as a marker of oligodendrocytic phenotype, since in humans, its appearance follows postmitotic oligodendrocytic commitment and the initiation of terminal differentiation (Kirschbaum et al., 1994; Armstrong et al., 1992; Roy et al., 1999). In contrast to the statin-associated increment in O4-defined oligodendrocytes, both the number and relative proportion of A2B5 progenitors dramatically declined in simvastatin-treated cultures (18.0 ± 5.1% to 2.7 ± 0.7%). When the relative proportions of A2B5 and O4 cells were plotted as a function of dose, we found the ratio of O4/A2B5 cells increased from <0.05 to 19 ± 4.6 with simvastatin treatment (Fig. 1). When the regression lines of O4 and A2B5 were plotted against simvastatin dose, their respective slopes significantly differed (Fig. 1E, P < 0.001 by 2-tailed t-test on three paired samples). ANOVA of the incidence of A2B5 and O4 cells as a function of simvastatin dosage revealed a significant, dose-dependent suppression of A2B5 phenotype (P < 0.01, F = 10.6) and relative expansion of O4 cells (P < 0.0001, F = 55.9 [2, 4 d.f.]). Post hoc pair-wise analysis showed that all simvastatin dosages (5–30 μM) significantly increased the proportion of O4 cells, and that simvastatin dosages >5 μM significantly depleted the A2B5 population (P < 0.05).

Pravastatin, a hydrophilic statin with an otherwise analogous set of actions as simvastatin, exerted a similar influence on oligodendrocytic differentiation by cultured GPCs. Seven days of treatment with 100 μM of pravastatin increased the proportion of O4 cells from 10.8 ± 4.0% to 47.3 ± 3.6% (Fig. 3A) (n = 4, P < 0.0001; F = 13.3; one-way repeated measures ANOVA). ANOVA with post hoc pairwise testing revealed that pravastatin doses above 10 μM significantly influenced the relative proportions of both A2B5 and O4 cells (P < 0.05). The ratio of O4/A2B5 cells rose from <1.5 to 4 ± 1.3 with exposure to pravastatin (P < 0.01; by one way ANOVA) (Fig. 3B). Similarly, the slopes of A2B5 and O4 regression lines were significantly different (P < 0.0001 by two-tailed t-test on four-paired samples). Importantly, pravastatin was not associated with observable toxicity over the tested doses spanning 0–100 μM, over which the rate of incorporation of propidium iodide (PI), a marker of irreversible membrane damage, varied little, with a range of 10.5–12.0%.

Fig. 2. Simvastatin and pravastatin reduce rate of the proliferation of adult glial progenitor cells (GPCs) while not affecting cell survival. Adult human GPCs were treated with either simvastatin or pravastatin for 7 days in vitro. Dose–response curve of the proportion of cells incorporating PI (± SEM, n = 3–4) or stained for BrdU (range shown, n = 2) following treatment with either simvastatin (A) or pravastatin (B).

The mitotic fraction of A2B5 GPCs was assessed by exposing the cultures to BrdU for 24 h prior to fixation at 7 DIV, then assessing the fraction of BrdU cells among all A2B5 cells, as a function of simvastatin (C) or pravastatin (D) dosage (range shown, n = 2). Mevalonate (Mev) was included to assess the specific contribution of HMG-CoA inhibition to any statin-associated treatment effects.

GLIA
Statins Reduced A2B5⁺ Cell Proliferation Without Affecting Survival

The dose-dependent changes in the relative proportions of oligodendrocytes and their progenitors in simvastatin-treated cultures were not due to any dose-dependent or phenotype-selective toxicity, over the dose range assessed. After a week’s exposure to 30 μM simvastatin, only 10.7 ± 0.7% of cells incorporated propidium iodide (PI) as a marker of cell death. This level of cell death did not differ from the 10.3 ± 1.5% incidence of PI incorporation noted in untreated controls (Fig. 2A). Thus, the simvastatin-associated increment in oligodendrocytes could not be attributed to any selective toxicity to progenitor cells. However, simvastatin doses exceeding 30 μM were associated with both dose and duration-dependent loss of GPCs (not shown), so that a maximum dose of 30 μM was used in these experiments.

Statin treatment did not influence the mitotic fraction of cultured glial progenitors; the proportion of BrdU⁺ cells did not differ as a function of either simvastatin or pravastatin treatment. Whereas 45.3% of untreated cells incorporated BrdU in baseline media (0 μM), 46.3% of cells in matched cultures supplemented with 30 μM simvastatin did so (range: 43.2–47.5% untreated, 45.0–47.7% simvastatin treated, n = 2 patients) (Fig. 2A). Similarly, the proportion of mitotic, BrdU⁺ cells was not affected by pravastatin (42.3–51.4% at baseline vs. 40.7–48.5% after a week in 100 μM pravastatin, n = 2 patients) (Fig. 2B). However, the proportion of BrdU⁺ cells among those cells persisting as A2B5⁺-expressing progenitors fell with statin treatment, such that whereas 21.6% of A2B5⁺ GPCs incorporated BrdU in control wells, only 9.2% of A2B5⁺ cells treated with 30 μM simvastatin did so (range: 18.9–24.2% untreated, 8.8–9.6% simvastatin treated, n = 2) (Fig. 2C). Similarly, pravastatin reduced the proportion of BrdU⁺ cells among all A2B5⁺ cells from 19.3% in untreated cultures to 10.5% in those exposed to 100 μM pravastatin (range: 18.5–20.2% untreated, 9.1–11.8% pravastatin treated, n = 2) (Fig. 2D). In each case, the fall over time in the proportion of BrdU⁺ cells among the total A2B5⁺ cell population appeared to reflect the departure of mitotic cells from the progenitor pool, with their terminal postmitotic differentiation as astrocytes and oligodendrocytes. Both of these statin-induced effects showed a significant correlation with the log₁₀ dosage of drug (simvastatin, r² = 0.97, P < 0.05; pravastatin, r² = 0.98, P < 0.01).

Statin-Induced Oligodendrocytic Differentiation Required Inhibition of Mevalonate Synthesis

We next asked whether the effects of statins were specifically mediated via HMG-CoA reductase inhibition, and hence mevalonate synthesis inhibition. To this end, we added excess mevalonate, the downstream product of HMG-CoA reductase to statin-treated cultures. When human GPCs were additionally challenged by 100 μM of mevalonate, together with either 30 μM simvastatin or 100 μM pravastatin, the statin-induced effects on the incidence of both A2B5⁺ and O4⁺ cells were abrogated, with levels of O4⁺ and A2B5⁺ cells reverting to those of null conditions (Fig. 1C,D, 3B). In addition, treatment with mevalonate reversed the statin-associated suppression of A2B5⁺ cell turnover, such that the proportion of BrdU⁺ cells among all A2B5⁺ cells returned to the level of untreated controls (Fig. 2C,D).

Statins Induced the RXR Nuclear Co-receptor PPARγ in GPCs

Our gene expression-based model suggested that the statin-mediated inhibition of HMG-CoA reductase might induce the nuclear co-receptor PPARγ. Since PPARγ agonists have been associated with oligodendrocytic differentiation (Roth et al., 2003), reflecting their characteristic prodifferentiative actions in a variety of cell types (Walczak and Tontonoz, 2002), we postulated that the de-repression of PPARγ transcription afforded by statin-mediated HMGCR inhibition might be associated with oligodendrocytic phenotypic differentiation. In support of this hypothesis, Antel and coworkers have recently
reported oligodendrocytic process elaboration in response to simvastatin (Miron et al., 2007). To test this postulate, we used real-time qPCR to quantify changes in PPAR5 mRNA following pravastatin treatment of human GPCs. Human GPCs treated with 100 μM pravastatin for 5–7 days showed a significant, 2.50 ± 0.36 fold increase in PPAR5 mRNA (paired t-test, \( P = 0.025 \), \( n = 4 \) patients). Addition of 100 μM mevalonate to pravastatin-treated cultures prevented significant PPAR5 mRNA induction, such that PPAR5 expression did not differ from untreated controls (1.51 ± 0.24, paired t-test \( P = 0.130 \), \( n = 4 \)). Thus, pravastatin treatment induced PPAR5 in cultured adult human GPCs. This effect required pravastatin’s inhibition of mevalonate synthesis, in that significant statin-associated induction of PPAR5 was inhibited by mevalonate addition.

To examine the effect of statin treatment on the transcription of other human GPC cholesterol regulators, we performed qPCR for other sterol pathway components on 10^6 cells treated for a week with either pravastatin alone or pravastatin together with mevalonate. The intracellular cholesterol regulators INSG1 and LDLR were both expressed at significantly lower levels following 100 μM pravastatin treatment (Table 2). The down-regulation of INSG1 by pravastatin was especially notable, since INSG1 may act as a negative regulator of PPARγ synthesis, suggesting that the statin-associated fall in INSG1s served to de-repress PPARγ transcription. Interestingly, HMGCGR mRNA expression was also down-regulated by pravastatin, suggesting that sterol synthesis inhibition might act to negatively regulate HMGCGR transcription. Among those cultures treated with mevalonate as well as pravastatin, the expression of LDLR normalized to null control levels, whereas the expression of INSG1 and HMGCGR mRNAs rose to levels exceeding those of their null controls, by 2.68 and 1.41-fold, respectively (paired t-test, \( P < 0.05 \), \( n = 4 \); Table 2). Thus, statin treatment suppressed the transcription of a number of critical sterol pathway components, including transcripts such as INSG1, that are critically important to intracellular sterol signaling.

### Statin-Induced PPARγ Transcription Was Associated with Oligodendrocyte Differentiation

We next asked if the statin-associated potentiation of oligodendrocyte differentiation required the induction of PPARγ. To this end, we exposed adult human GPCs to the PPARγ-selective and irreversible antagonist, GW9662 (Leesnitzer et al., 2002), and then challenged the cells with 100 μM pravastatin. We noted that pravastatin expanded the O4 population (11.7 ± 0.9% vs. 34.0 ± 1.2%, \( n = 3 \), paired t-test \( P < 0.001 \)) and depleted the A2B5 population (30.3 ± 0.9% vs. 19.7 ± 2.6%, \( n = 3 \), paired t-test \( P < 0.05 \)) (Fig. 4A,B). In contrast, treatment with GW9662 suppressed pravastatin-induced oligodendrocyte differentiation restoring the relative proportions of O4+ and A2B5+ cells to those of naive controls not exposed to statins (Fig. 4C). The GW9662-mediated suppression of pravastatin-associated oligodendrocyte differentiation was specific to the statin-treated cultures, since GW9662 given at a 10-fold higher dose than that used to block pravastatin’s effects elicited no changes in either the numbers or relative proportions of O4+ and A2B5+ progenitors (Fig. 4D). These results further suggest that statin treatment potentiated oligodendrocytic differentiation via PPARγ-mediated signaling and transcriptional activation.

### DISCUSSION

The statin drugs are one of the most widely used classes of drugs in the Western world. As of 2005, over 12 million statin prescriptions are issued every month in the US alone (Consumers Union, 2006), to patients receiving largely chronic statin therapy for hypercholesterolemia, or for prophylaxis of arteriopathy in the setting of known diabetes or cardiovascular disease. Remarkably, almost 10% of the American adult population has been prescribed a statin, as has over a quarter of the over-65 population (Consumers Union, 2006; Carroll et al., 2005). Moreover, additional indications for statin use may be on the horizon, as they are evaluated for the treatment of anti-inflammatory diseases, multiple sclerosis in particular.

In this study, we report that statin-mediated inhibition of sterol synthesis may be associated with the triggering of a prodifferentiative pathway that leads to the generation of oligodendrocytes from adult human glial progenitor cells. In particular, we found that statin-induced oligodendrocyte differentiation was mediated via inhibition of HMG-CoA reductase activity, in that it was completely antagonized by supplementing the culture media with excess mevalonate. Furthermore, statin-induced oligodendrocyte differentiation was associated with upregulated PPARγ expression, and PPARγ inhibition blocked the statin-associated oligodendrocyte differentiation. Together, these results confirmed our
genomics-based hypothesis that statin treatment might potentiate oligodendrocyte differentiation (Fig. 5). In this regard, it is also important to note that statin inhibition of mevalonate synthesis may also influence oligodendrocytic maturation and process elaboration through isoprenylation of signaling molecules like the Rho GTPases. In particular, elegant studies by Miron et al. have revealed that a statin-mediated inhibition of isoprenylation may potentiate oligodendrocytic morphological maturation and process extension by interfering with the isoprenylated RhoA GTPase, and hence suppressing Rho kinase activity (Miron et al., 2007). In contrast, we report here that statins may also potentiate oligodendrocyte maturation by triggering oligodendrocytic fate commitment by glial progenitor cells, through a distinct pathway leading to the PPARγ-dependent differentiation of these otherwise uncommitted progenitor cells. In addition, a co-operative interaction between statin-triggered PPARγ signaling and statin-mediated inhibition of Rho GTPase may also be operative, as has already been described in peripheral blood monocytes (Ruiz-Velasco et al., 2004). In this regard, PPARγ activation in rat aortic smooth muscle cells has been reported to inhibit Rho/Rho kinase activity (Wakino et al., 2004), suggesting the possible feedback inhibition of Rho kinase by PPARγ-dependent signals. These observations suggest the importance of coordination between statin-induced PPARγ- and Rho/Rho kinase inhibition in the differentiation of oligodendrocytes from parenchymal glial progenitor cells.

Both lipophilic simvastatin and hydrophilic pravastatin exerted similar effects on the differentiation of adult glial progenitor cells. We noted statin-associated toxicity only with simvastatin, similar to previous observations made in rat neuroblasts (Garcia-Roman et al., 2001). In cultured glia from the human fetal brain, simvastatin exhibited dose-dependent induction of fiber outgrowth, whereas pravastatin did not (Miron et al., 2007), suggesting that the lesser membrane permeance of pravastatin might limit its cellular effects in glia in vitro, just...
as it does in vivo (Saheki et al., 1994). Yet pravastatin has been shown to induce significant neurite outgrowth in rat cultured hippocampal neurons via a RhoA GTPase-dependent mechanism, suggesting that pravastatin can indeed modulate neural cellular physiology (Pooler et al., 2006). In the liver, an ATP-dependent active transport mechanism is responsible for pravastatin uptake (Nezasa et al., 2003), suggesting that an analogous process might explain pravastatin actions on cultured neural cells. Indeed, organic anion transporters able to transport pravastatin have been described in the adult brain (Kusuhara et al., 1999; Takeda et al., 2004). In this regard, we found that MCT2 (SLC16A7) a monocarboxylic acid transporter that shuttles statin acids (Tsui et al., 1993) was significantly expressed by adult GPCs in our microarray studies (Sim et al., 2006). Accordingly, pravastatin has been found at significant levels in the brains of treated animals (Johnson-Anuna et al., 2005; Thelen et al., 2006). Importantly, the statin-associated induction of oligodendrocyte differentiation that we observed in culture occurred at levels obtainable in the brains of normal patients treated by high-dose statins.

Importantly, the increase in oligodendrocyte differentiation was attended by a concomitant diminution in A2B5-defined GPCs, suggesting that statin treatment might be associated with a progressive depletion of uncommitted GPCs. Thus, although the oligoneogenic effect of statins may contribute to their beneficial effects on central remyelination after acute injury—previously ascribed to their anti-inflammatory actions—this benefit may be accompanied by a depletion of central glial progenitor pools. In the setting of chronic statin therapy, we might then predict a cumulative depletion of competent GPCs that may sharply diminish the capacity of the adult brain parenchyma for both homeostatic and reactive oligodendrocyte production. This in turn might diminish the adult brain’s ability to respond adaptively to aging and injury. In particular, the induction of precocious differentiation may result in progenitor depletion in situations where compensatory oligodendroglial production might otherwise occur, such as following a demyelinating or vascular insult.

The implications of such statin-accelerated oligodendrocytic differentiation are manifold. Statins are under consideration for the treatment of multiple sclerosis and the inflammatory demyelinations largely on the basis of their anti-inflammatory effects. Yet if statin treatment is attended by progenitor depletion in vivo, then one might expect that statin treatment might serve to hasten the loss of competent oligodendrocyte progenitor cells during cycles of inflammatory demyelination. If so, then early statin treatment might actually accelerate the loss of ambient progenitor pools in MS, impeding myelin repair, while conceivably speeding the progression from relapsing-remitting to secondary progressive MS.

In addition, given the extended periods of time over which patients are treated with statins, commonly for decades after an initial diagnosis of hypercholesterolemia, the homeostatic self-renewal of GPCs in adult brain may be impaired. As such, statins may deplete the population of progenitors available for later recruitment during aging or in response to minor injury or vascular insult. This in turn suggests the possibility of premature cognitive decline by patients subjected to chronic statin treatment (Muldoon et al., 2004). In this regard, although prospective studies to date have failed to demonstrate a causal relationship between statin use and dementia (Wagstaff et al., 2003), the co-morbidities of hypercholesterolemic patients requiring chronic statin use might obscure such a relationship, while the time frames of these studies may have been too short for such an association to become manifest in any event (Cutler et al., 1995; Golomb et al., 2004). In this context, our results suggest the need for awareness of the possible toxicities accruing to long-term statin use, and identify one such potential toxicity, the premature differentiation and attendant long-term depletion of oligodendrocyte progenitor cells of the adult brain.

REFERENCES


