

Differential effects of simvastatin and pravastatin on expression of Alzheimer's disease-related genes in human astrocytes and neuronal cells

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Abstract Inhibitors of HMG-CoA reductase (statins) are widely used medications for reduction of cholesterol levels. Statin use significantly reduces risk of cardiovascular disease but has also been associated with lower risk of other diseases and conditions, including dementia. However, some reports suggest that statins also have detrimental effects on the brain. We provide evidence that simvastatin and pravastatin have significantly different effects on expression of genes related to neurodegeneration in astrocytes and neuroblastoma (SK-N-SH) cells in culture. Simvastatin significantly reduced expression of *ABCA1* in astrocytes and neuroblastoma cells (by 79% and 97%, respectively; both $P < 0.001$). Pravastatin had a similar but attenuated effect on *ABCA1* in astrocytes ($-54%$, $P < 0.001$) and neuroblastoma cells ($-70%$, $P < 0.001$). Simvastatin reduced expression of apolipoprotein E in astrocytes ($P < 0.01$). Furthermore, both statins reduced expression of microtubule-associated protein tau in astrocytes ($P < 0.01$), while both statins increased its expression in neuroblastoma cells ($P < 0.01$). In SK-N-SH cells, simvastatin significantly increased cyclin-dependent kinase 5 and glycogen synthase kinase 3 β expression, while pravastatin increased amyloid precursor protein expression. Our data suggest that simvastatin and pravastatin differentially affect expression of genes involved in neurodegeneration and that statin-dependent gene expression regulation is cell type specific.—Dong, W., S. Vuletic, and J. J. Albers. Differential effects of simvastatin and pravastatin on expression of Alzheimer's disease-related genes in human astrocytes and neuronal cells. 2009. *J. Lipid Res.* 50: 2095–2102.

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Statins are competitive inhibitors of HMG-CoA reductase, a rate-limiting enzyme for cholesterol synthesis, and have been widely used as plasma cholesterol lowering drugs in dyslipidemic patients. Many large-scale clinical intervention trials have demonstrated that statins significantly lower morbidity and mortality, particularly due to reduction in cardiovascular events (1). Furthermore, numerous studies in recent years suggested that prolonged use of statins is associated with lower risk of other diseases, including dementia due to Alzheimer's disease (AD) (2–6). These findings led to recommendations for significant increase in use of statins and prompted several clinical trials where statins were tested in people who already have AD. However, data published so far suggest that use of statins in AD patients led to mixed outcomes (7–12), somewhat diminishing the initial hope that statins may not only prevent processes leading to AD, but also retard the disease when it is already clinically apparent. Furthermore, a report by Algotsson and Winblad (13) suggested that patients with AD may have increased susceptibility to statin-induced adverse effects.

Additionally, although less often considered as an issue with use of statins, particularly those that cross the blood-brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier, is the fact that some patients develop symptoms of dementia as a result of statin therapy, which improve or disappear upon statin withdrawal (14–16). These findings are rarely reported, and many physicians tend to regard patient-reported memory impairment, particularly in the

Abbreviations: AD, Alzheimer's disease; apoE/APOE, apolipoprotein E; APP, amyloid precursor protein; BBB, blood-brain barrier; CDK5, cyclin-dependent kinase 5; CSF, cerebrospinal fluid; DAB1, Disabled 1; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; GSK3 β , glycogen synthase kinase 3 β ; MAPT, microtubule-associated protein tau; PLTP, phospholipid transfer protein.

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elderly, as an age-related, rather than a therapy-related issue. Despite the fact that memory impairment is rarely reported as an adverse effect of statin therapy, the statin-induced memory problems are real, potentially causing a substantial amount of concern and confusion to the patients. The effects of statins on the brain are far less well understood than their effects on other target organs.

In a previously reported randomized pilot clinical trial in statin-naïve hypercholesterolemic patients with no measurable cognitive impairment, we have shown that simvastatin, a lipid-soluble statin that easily crosses the BBB, and pravastatin, a hydrophilic statin, which does not readily cross the BBB, have differential effects on some AD-related markers in human CSF (17, 18). Simvastatin was associated with a statistically significant reduction in CSF levels of tau phosphorylated at Thr-181 (pTau_{Thr181}) and an increase in CSF phospholipid transfer protein (PLTP) activity. Levels of CSF apolipoprotein E (apoE) were significantly reduced in patients using pravastatin, and there was a trend for CSF apoE reduction in patients on simvastatin. Due to the different BBB transfer of these two statins, the observed differences may have been due to the intrathecal availability of individual statins or due to different effects of these statins on the brain cells. Therefore, to evaluate direct effects of lipophilic and hydrophilic statins on brain cells, we performed an in vitro study using primary human astrocytes and neuroblastoma (SK-N-SH) cells and assessed the effects of simvastatin and pravastatin on expression of *ABCA1*, *APOE*, *PLTP*, microtubule-associated protein tau (*MAPT*), and some of the genes related to tau phosphorylation, Disabled 1 (*DABI*), cyclin-dependent kinase 5 (*CDK5*), and glycogen synthase kinase 3 β (*GSK3 β*), and amyloid precursor protein (*APP*), the genes functionally associated with AD.

MATERIALS AND METHODS

Materials

Simvastatin was a gift from Merck (Whitehouse Station, NJ). Pravastatin, mevalonolactone, geranylgeranyl pyrophosphate (GGPP), and farnesyl pyrophosphate (FPP) were obtained from Sigma-Aldrich (St. Louis, MO). Kits used in RNA isolation were obtained from Qiagen (Valencia, CA). Cell culture media were obtained from Lonza (Walkersville, MD), collagen solution (Type I, from calf skin) from Sigma-Aldrich, and FBS with low endotoxin levels from HyClone (Logan, UT). Protein isolation solution, PhosphoSafe, was from Calbiochem/EMD Chemicals (San Diego, CA). Protease inhibitor cocktail, Halt, and growth factors used in tissue culture were obtained from Invitrogen (Carlsbad, CA). Gels, sample, and running buffers were obtained from Bio-Rad (Hercules, CA). Phospholipids for preparation of liposomes used in phospholipid transfer assay were obtained from Sigma-Aldrich, and radioactive phosphatidylcholine from Perkin-Elmer Life Sciences/NEN (Boston, MA). Cholesterol measurement kit, Amplex Red, and the RiboGreen® RNA Quantitation Kit were obtained from Molecular Probes (Eugene, OR).

Activation of simvastatin

Simvastatin needs to be activated by opening of the lactone ring before use in cell culture. We used the protocol provided by

Merck. Briefly, eight milligrams of simvastatin (0.019 mM) were dissolved in 0.2 ml of 100% ethanol, with subsequent addition of 0.3 ml of 0.1 N NaOH. The solution was heated at 50°C for 2 h in a sand bath and then neutralized with HCl to pH 7.2. The resulting solution was brought to a final volume (1 ml) with distilled water, and aliquots were stored at -80°C until use.

Preparation of mevalonate from mevalonolactone

One gram of mevalonolactone was mixed with 9 ml of sterile water. Using 1 N NaOH, pH was adjusted to 7.4. Aliquots were stored at -80°C until use.

Cell culture

Primary human astrocytes from four different donors (gift from Dr. Möller, Department of Neurology, University of Washington, Seattle) were prepared from tissue obtained from legally aborted fetuses (Birth Defects Laboratory, University of Washington, Seattle) using previously described methodology (19). Primary human astrocytes and SK-N-SH neuroblastoma cell line (ATCC) were plated on 6-well plates and grown in DMEM supplemented with 5% or 8% fetal calf serum (HyClone, Logan, UT), respectively, at 37°C, 5% CO₂ until 80% confluent. For measurement of gene expression levels at baseline, cells were just washed and RNA was prepared and assayed as outlined below. Baseline gene expression levels in astrocytes were measured in primary human astrocytes obtained from two donors. For experimental purposes, cells were incubated under serum-free conditions. Primary human astrocytes used for the reported results were obtained from four donors. Based on preliminary time-dependent studies, 48-h incubation was used for all of the reported experiments. Based on the dose-response studies, a majority of our experiments were conducted using the following concentrations of active compounds: simvastatin at 5 μ M, pravastatin at 10 μ M, mevalonate at 50 μ M, and GGPP and FPP at 10 μ M. Following incubation, cells were extensively washed to remove dead cells and cell debris and prepared for further analyses.

Protein isolation, electrophoresis, and Western blotting

Cells were scraped and cell protein isolated using Phospho-Safe protein isolation solution (EMD Chemicals) in the presence of protease inhibitors (Halt protease inhibitor cocktail; Invitrogen), according to the manufacturer's instructions and stored at -80°C until use.

Cell proteins were subjected to denaturing SDS-PAGE and Western blot analysis. Samples were loaded on 4–12% gel (Bio-Rad Bis-Tris Criterion XT) for SDS-PAGE and, following electrophoresis, transferred to nitrocellulose membrane using Bio-Rad semidry transfer. Blots were incubated overnight with primary antibodies at 4°C with agitation in 5% milk/TBS-Tween 20. Loading controls were evaluated using β -actin. The blots were incubated with the appropriate TrueBlot secondary antibody for 2 h at room temperature. The membranes were developed using West Femto SuperSignal chemoluminescent kit and scanned using Kodak CF400 imaging system.

Phospholipid transfer activity assay

PLTP-mediated phospholipid transfer activity was assessed using PLTP phospholipid transfer activity assay (20). Ten microliters of conditioned media were assayed for the ability to transfer C14-labeled phospholipid from donor liposome particles to the acceptor particles (plasma HDL without measurable PLTP activity). All values were adjusted for the background transfer, and PLTP activity was calculated as percentage of phospholipid transfer per microliter of conditioned medium per hour (%/ μ l/h).

RNA isolation

RNA was isolated using the Qiagen RNEasy kit according to the manufacturer's instructions. To remove trace genomic DNA, samples were treated with DNase and DNA-Shredder kit (Qiagen). Following assessment of 260/280 nm ratio by spectrometry, RNA samples were stored at -80°C until use. Total RNA was quantified on the Mx4000® Multiplex QPCR System (Stratagene, La Jolla, CA) using the RiboGreen® RNA Quantitation Kit (Molecular Probes). All samples obtained from cells grown under chosen experimental conditions (5 μM simvastatin and 10 μM pravastatin) had highly similar total RNA yield ($46.8 \pm 3 \mu\text{g}/\text{ml}$), and we did not observe significant cell death under these conditions.

Quantitative PCR

Quantitative PCR was performed on an Mx4000® Multiplex QPCR System with samples loaded in triplicate using 40 ng of total RNA. Quantitative PCR was run in a 10 μl reaction using SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA) (5 μl 2 \times Master Mix, 400 nM each primer, 0.5 units of StrataScript RT, and 0.5 units of RNase Block) with PCR cycling conditions of 48°C for 30 min, 95°C for 10 min, and 40 cycles at 95°C for 15 s, 60°C for 1 min. After each assay, a dissociation curve was run to confirm specificity of all PCR amplicons. Resulting C_t values were converted to nanograms, normalized to S18 mRNA and total RNA, and expressed as the average of triplicate samples \pm SD. Pooled sample total RNA was used for standard curves in 1:2 serial dilutions.

Primers were from Operon (Huntsville, AL) and designed using Applied Biosystems Primer Express 2.0 software. The primers and probes used for each gene are listed in **Table 1**.

Cholesterol measurement

Levels of intracellular cholesterol were evaluated using the Amplex Red kit (Molecular Probes) according to the manufacturer's instructions. Cells were lysed using the MEM-PER kit (Pierce), and cell lysates were evaluated for intracellular cholesterol in the presence of cholesterol oxidase and cholesterol esterase, with appropriate cholesterol standards and positive and negative controls. Samples were incubated for 30 min at 37°C and 60 rpm, cooled for 5 min at room temperature, and read at excitation/emission of 530/590 nm in Bioscan fluorescent plate reader. Data were analyzed using Hidex software, corrected for cell protein, and expressed as picograms of cholesterol per microgram of cell protein ($\text{pg}/\mu\text{g}$).

Statistical analyses

Statistical analyses were performed with Statistica for Windows (StatSoft, Tulsa, OK). Differences were evaluated using Mann-Whitney U-Test and *t*-test. *P* values <0.05 or <0.01 (for multiple comparisons) were considered statistically significant.

RESULTS

Baseline expression

Baseline expression levels of AD-related genes, prior to removal of serum supplementation, in primary human astrocytes and SK-N-SH cells are shown in **Table 2**. Comparison between baseline gene expression and control samples following 48-h incubation in serum-free media suggests that astrocytes and SK-N-SH cells respond differently to serum removal. In astrocytes, serum removal reduced expression of genes involved in removal of cellular lipids, such as *ABCA1* and *PLTP*. Surprisingly, there was no effect of serum removal on expression of *APOE*. In contrast, removal of serum led to an increase in *ABCA1* and *PLTP* expression in neuroblastoma cells (**Table 2**). Baseline gene expression in primary human astrocytes was evaluated in astrocytes obtained from two sources. No significant difference in baseline gene expression was found between two astrocytic cultures (data not shown). Spearman correlation coefficients for baseline levels of different genes in primary human astrocytes and SK-N-SH cells suggest that gene expression levels of some genes, for example, *APOE* and *MAPT* in astrocytes, are highly correlated and possibly regulated by the same pathway under normal growth conditions (**Table 3**).

Dose response

Dose-response studies indicate that a low dose of statins (1 μM) has no statistically significant effect on gene expression in both primary human astrocytes and neuroblastoma cells. For example, simvastatin dose of 5 μM and pravastatin dose of 10 μM achieved maximum effect on *ABCA1* gene expression (**Fig. 1**). Similar results were obtained for other tested genes (data not shown). Therefore,

TABLE 1. List of primers used in this study

Gene	Accession No.	Primer Name	Primer	Amplicon (nt)
ABCA1	NM005502	hABCA1-3296F	TGGTTCTATGCCCGCTTGA	176
		hABCA1-3471R	ACCTTAGATCCCCGACAAAG	176
PLTP	NM006227	hPLTP-289F	TCACAGAGCTGCAACTGACATCT	81
		hPLTP-369R	AGGAGGCATTGGTGATTTGAA	81
APOE	NM000041	hApoE-145F	AGCAAGCGGTGGAGACAGA	91
		hApoE-235R	AAGCGACCCAGTGCCAGTT	91
MAPT	NM005910 variants 1–4	hMAPT-4038F	CAGGGATTGGGATGAATTGC	113
		hMAPT-4150R	TCTGGTCAAGGCTTTGGGAA	113
CDK5	NM004935	hCDK5-94F	GGAAGGCACCTACGGAACTGT	101
		hCDK5-194R	GCACACCCTCATCATCGTCAT	101
GSK3 β	NM002093	hGSK3B-1526F	TCCACCTGAACAGTCCCCGA	81
		hGSK3B-1606R	CGTGACCAGTGTGCTGAGTG	81
APP	NM201414	hAPP-373F	GATGCCCTTCTCGTTCCTGA	86
		hAPP-458R	ACGGTGTGCCAGTGAAGATG	86
DAB1	NM021080	hDAB1-798F	TACAAAGCCAAATTGATCGGG	87
		hDAB1-884R	GCCCTTGAGTTTCATCATGGA	87

h, human; F, forward; R, reverse.

TABLE 2. Expression of Alzheimer's disease-related genes in primary human astrocytes and SKN-SH cells at baseline and following 48-h incubation in serum-free media

Gene	Astrocytes		SKN-SH	
	Baseline	48-h Incubation	Baseline	48-h Incubation
ABCA1	3.77 ± 0.3	1.43 ± 0.4 ^a	0.51 ± 0.1	1.42 ± 0.2 ^a
PLTP	2.46 ± 0.3	1.08 ± 0.3 ^a	0.84 ± 0.1	1.15 ± 0.3 ^a
APOE	0.86 ± 0.1	0.94 ± 0.2	NA	NA
MAPT	0.65 ± 0.2	1.18 ± 0.2 ^a	0.75 ± 0.1	1.16 ± 0.4 ^a
CDK5	0.26 ± 0.0	0.97 ± 0.3 ^a	0.90 ± 0.1	0.91 ± 0.2
GSK3β	1.08 ± 0.1	0.93 ± 0.1 ^a	0.88 ± 0.1	1.01 ± 0.2 ^a
APP	2.21 ± 0.2	1.01 ± 0.2 ^a	0.75 ± 0.1	0.96 ± 0.2 ^a
DAB1	6.90 ± 0.6	0.88 ± 0.2 ^a	0.13 ± 0.0	0.74 ± 0.1 ^a

Baseline levels in primary human astrocytes were measured in samples obtained from two donors, while 48-h incubation data were obtained from samples obtained from four donors. Data are shown as mean ± SD of gene/18S. NA, not applicable.

^a $P \leq 0.01$ by *t*-test.

we used a simvastatin dose of 5 μM and a pravastatin dose of 10 μM in our subsequent experiments.

To evaluate biological activity of the chosen statin concentrations, we measured cholesterol levels in cells incubated with simvastatin and pravastatin (Fig. 2). As expected, biological effects of 5 μM of simvastatin and 10 μM of pravastatin on cholesterol levels were very similar. Astrocytes were more resilient to statin effect, with 47–49% reduction in intracellular cholesterol concentration in cells incubated with either statin. The same doses of statins had more profound effect on intracellular cholesterol in SKN-SH cells, with 92–97% reduction in intracellular cholesterol. These data suggest that the dose of statins used for the majority of our experiments has a similar effect on cholesterol reduction and that reduction of cholesterol in the presence of statins in astrocytes is significantly smaller than in SKN-SH cells.

Gene expression and statins

Statins induced significant changes in expression of *ABCA1*. In the presence of simvastatin, *ABCA1* expression

was highly significantly reduced in primary human astrocytes and nearly abolished in neuroblastoma cells (Fig. 3). In contrast, pravastatin had a somewhat lesser effect on *ABCA1* expression despite a biologically equivalent dose of pravastatin (10 μM), leading to a smaller reduction in *ABCA1* expression in both astrocytes and neurons (Figs. 1 and 3; Table 4). Addition of mevalonate to cells exposed to statins partially reversed the statin effects in both cell types. Partial reversal of the simvastatin effect following supplementation with mevalonate suggests that the observed reduction in *ABCA1* expression is at least partially related to reduced cholesterol synthesis. Addition of GGPP or FPP to cells exposed to statins had no significant effect on *ABCA1* expression in either cell type.

Incubation of astrocytes with simvastatin led to the expected decrease in *APOE* expression (Table 4). In contrast, pravastatin had a variable effect on *APOE* expression in astrocytes. Supplementation of astrocytes with mevalonate reversed the observed simvastatin-induced effects, while GGPP or FPP had variable effects on expression of *APOE* in primary human astrocytes.

Simvastatin and pravastatin significantly decrease expression of *PLTP* in primary human astrocytes. A similar effect was observed in *PLTP* phospholipid transfer activity, with both statins reducing *PLTP* phospholipid transfer activity by approximately 30% ($P < 0.05$). In neuroblastoma cells, only simvastatin had a significant effect on *PLTP* expression (Table 4). Statin effects on *PLTP* expression in both cell types were reversed by mevalonate supplementation. GGPP and FPP partially reversed simvastatin effect in SKN-SH cells but had no effect on statin-induced decrease in *PLTP* expression in astrocytes.

Incubation of astrocytes with simvastatin and pravastatin led to an approximately 60% and 40% decrease in expression of *MAPT*, respectively. Supplementation of astrocytes with mevalonate, GGPP, and FPP did not reverse the statin effect on *MAPT* expression in astrocytes, suggesting that the observed reduction is primarily due to a statin effect

TABLE 3. Spearman correlations for gene expression levels at baseline in primary human astrocytes (A) and SKN-SH cells (B)

A	ABCA1	PLTP	APOE	MAPT	CDK5	GSK3β	APP
ABCA1							
PLTP	0.534						
APOE	-0.020	0.335					
MAPT	-0.293	0.323	0.615 ^a				
CDK5	0.225	0.765 ^a	0.550	0.370			
GSK3β	0.486	0.645 ^a	0.439	0.103	0.514		
APP	0.728 ^a	0.620 ^a	0.214	0.031	0.553	0.505	
DAB1	0.524	0.329	0.066	-0.020	0.184	0.528	0.367
B	ABCA1	PLTP	MAPT	CDK5	GSK3β	APP	
ABCA1							
PLTP	-0.033						
MAPT	-0.100	0.483					
CDK5	0.550	0.017	-0.117				
GSK3β	0.424	0.339	-0.051	0.763			
APP	0.326	-0.184	-0.050	0.552	0.451		
DAB1	0.550	0.067	-0.217	0.883 ^a	0.644	0.435	

Data are based on gene expression adjusted for 18S (n = 18 for astrocytes and n = 9 for SKN-SH cells).

^a $P \leq 0.01$.

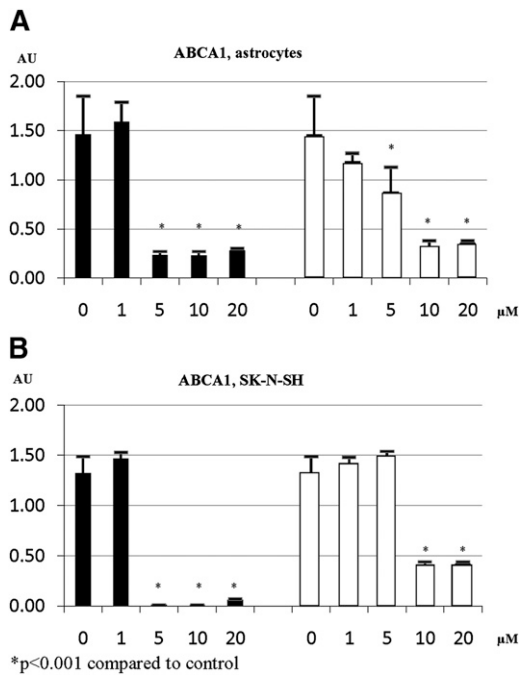


Fig. 1. ABCA1 gene expression in primary human astrocytes (A) and SK-N-SH cells (B) in response to different doses of simvastatin (closed bars) or pravastatin (open bars).

on pathways other than cholesterol synthesis. In contrast, incubation of neuroblastoma cells with both statins caused an increase in *MAPT* expression (Fig. 4A). Supplementation of neurons with mevalonate partially reversed the observed effects in neuroblastoma cells, suggesting partial

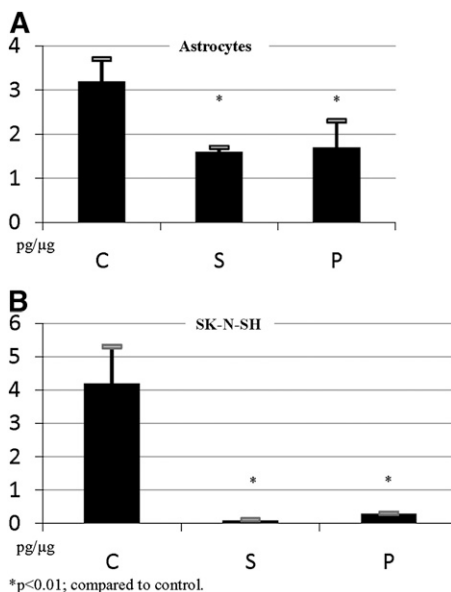


Fig. 2. Effects of statins on levels of intracellular cholesterol in astrocytes and SK-N-SH cells. Primary human astrocytes (A) and human neuroblastoma cells, SK-N-SH (B), were incubated without or with simvastatin (S; 5 μM) and pravastatin (P; 10 μM) for 48 h (n = 9 for each condition). Levels of intracellular cholesterol were measured using the Amplex Red kit (excitation/emission 530/590 nm) and expressed as picograms of cholesterol per microgram of total protein. C, control.

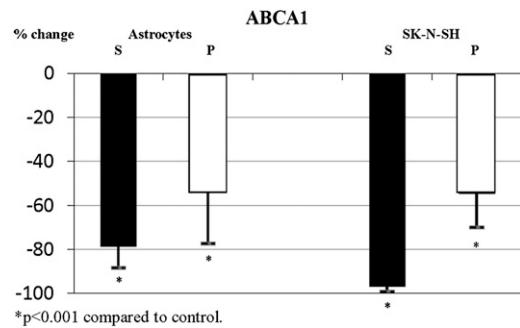


Fig. 3. Simvastatin and pravastatin significantly reduce expression of *ABCA1* in primary human astrocytes and SK-N-SH cells. Expression of *ABCA1* in primary human astrocytes and human neuroblastoma cells following incubation with simvastatin (closed bars, S; 5 μM) and pravastatin (open bars, P; 10 μM).

involvement of cholesterol synthesis pathway in regulation of *MAPT* expression in neuronal cells. These results were confirmed also at the protein level (Fig. 4B), indicating that statins oppositely affect astrocytes and neurons at the level of both gene and protein expression.

Furthermore, we evaluated the effects of statins on neuronal expression of *DABI*, *CDK5*, and *GSK3β*, proteins known to regulate tau phosphorylation in neurons (21, 22). Simvastatin significantly increased expression of *CDK5* and *GSK3β* in neuroblastoma cells but had no significant effect on *DABI*. In astrocytes, pravastatin moderately decreased expression of *DABI* and *CDK5* (Table 4). Supplementation of mevalonate reversed simvastatin effects on *GSK3β* but had no effect on expression of *CDK5* in neuronal cells.

Evaluation of *APP* expression in neurons suggested that simvastatin had no effect, while incubation with pravastatin led to a statistically significant increase in *APP* expression (Table 4). Neither statin affected *APP* expression in astrocytes. Similar results were seen at the protein level (data not shown).

TABLE 4. Effects of simvastatin (5 μM) and pravastatin (10 μM) on gene expression in primary human astrocytes and SK-N-SH cells

	Gene	Simvastatin	Pravastatin	
Astrocytes	ABCA1	-78.6 ± 9.8 ^a	-54.25 ± 23.1 ^a	
	PLTP	-31.5 ± 11.8 ^a	-42.9 ± 24.2 ^a	
	APOE	-23.1 ± 10.2 ^a	-26.9 ± 25.1	
	MAPT	-49.5 ± 4.9 ^a	-40.8 ± 3.8 ^a	
	CDK5	-6.4 ± 0.7	-14.6 ± 1.8 ^a	
	GSK3β	-3.7 ± 15.3	3.4 ± 5.0	
	APP	-4.3 ± 5.4	-1.6 ± 9.8	
	DABI	4.4 ± 24.7	-20.72 ± 11.1 ^a	
	SK-N-SH	ABCA1	-96.8 ± 2.4 ^a	-69.8 ± 15.5 ^a
		PLTP	-29.6 ± 6.1 ^a	-2.9 ± 21.2
MAPT		40.9 ± 11.1 ^a	19.3 ± 4.7 ^a	
CDK5		36.4 ± 11.8 ^a	1.5 ± 6.4	
GSK3β		20.4 ± 7.9 ^a	4.7 ± 9.9	
APP		16.6 ± 14.7	15.2 ± 4.6 ^a	
DABI		-10.9 ± 34.0	-2.2 ± 19.3	

Primary human astrocytes used in these experiments were obtained from four different donors. Data are presented as average percentage change ± SD in at least three consecutive experiments performed in triplicate and assayed in three technical replicates.

^a P < 0.05 represents statistically significant difference between control and statin data, by t-test, that was replicated in each individual experiment.

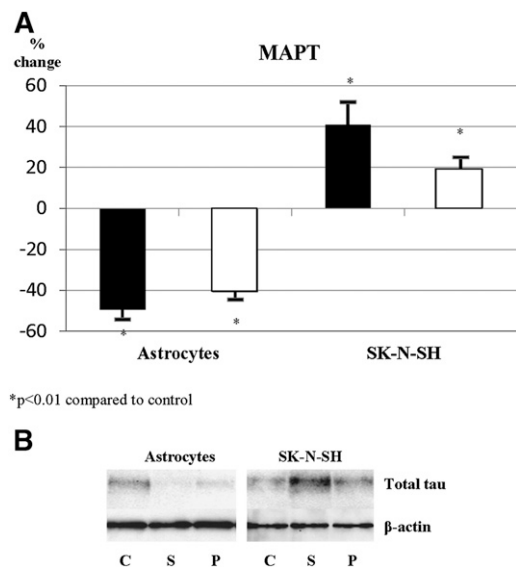


Fig. 4. Statin effect on expression of *MAPT* differs in primary human astrocytes and SK-N-SH cells. **A:** *MAPT* expression levels in primary human astrocytes and SK-N-SH cells incubated with simvastatin (5 μM, closed bars) and pravastatin (10 μM, open bars). **B:** Total tau protein in primary human astrocytes and SK-N-SH cells.

DISCUSSION

Data from our study indicate that simvastatin and pravastatin had differential effects on expression of genes relevant for AD development and that these effects are dependent not only on the type of statin but also on cell type. Generally, astrocytes were less susceptible to statin effect, probably due to a greater resilience in changes in cholesterol levels in response to statin treatment. Despite the identical concentrations of simvastatin and pravastatin, the effect on expression of *ABCA1* in astrocytes was significantly weaker compared with neurons. *ABCA1* regulates intracellular levels of cholesterol and phospholipids through regulation of cellular lipid efflux (23). Under the conditions of significantly reduced cholesterol synthesis, reduction in *ABCA1* expression would effectively reduce lipid efflux from neurons, thus preserving the existing intracellular cholesterol.

Astrocytes are the main source of lipoproteins in the brain, and *ABCA1* is one of the key *in vivo* determinants of secretion of the lipoproteins containing apoE (24, 25). ApoE is a major apolipoprotein in the brain, and under the normal conditions, all brain apoE is locally synthesized (26–28). Although the effects of statins on *APOE* expression in astrocytes were relatively modest compared with the effects on *ABCA1*, reduction in both *ABCA1* and *APOE* in the brain is likely to be detrimental in the long term. ApoE synthesis and secretion in glial cells is one of the critical processes for maintenance of the brain lipid homeostasis. ApoE-containing lipoproteins are the main lipid carriers in the brain, and binding of apoE to its receptors on the neuronal membrane is important not only for holoparticle lipoprotein uptake but also for cell signaling (26, 27, 29). Increasing the level of apoE in the brain generally has beneficial effects on numerous cell and tis-

sue processes, including membrane repair and memory (30–36).


Published studies suggest that neurons do not synthesize sufficient amounts of cholesterol needed for maintenance of their functional and structural stability but depend on lipid delivery from glial cells through glia-derived lipoproteins (37). The reduced lipoprotein delivery from astrocytes due to long-term statin therapy may not be sufficient to maintain structural and functional stability of neurons. Published studies report that astrocyte-derived cholesterol is essential for formation of synapses in neurons and that continuous cholesterol recycling is necessary for structural and functional stability of neurons and for synaptic transmission/long-term potentiation (37, 38). In vulnerable patients, the observed significant reduction in *ABCA1* expression combined with the reduction in expression of *APOE* and *PLTP* would reduce the level of circulating astrocyte-derived lipoproteins, thus significantly reducing availability of lipids necessary for neuronal membrane repair and for formation, maintenance, and function of synapses (30, 37, 38). Therefore, the long-term effects of statin therapy could lead to transient or permanent cognitive impairment in patients who already have low levels of brain cholesterol. This is primarily an issue in the elderly because brain cholesterol synthesis and levels tend to be reduced in people with advanced age, and cholesterol as well as phospholipid levels are particularly reduced in patients with AD (39–42). While the observed reduction in *ABCA1* and *APOE* may not be the only factors that contribute to a statin-induced cognitive impairment in vulnerable patients, it is likely a contributing factor that should be taken into account when deciding on dosage and type of statin used in individual patients.

Statins reduce *ABCA1* expression in both astrocytes and neurons, but the extent of the reduction was different between the two cell types. In contrast, statins significantly reduced *MAPT* expression in astrocytes but significantly increased its expression in neurons. These findings indicate that the nature of the *MAPT* expression regulation by statins is cell type dependent. Functional implications of these differences are currently unclear, although it could be argued that an increase in *MAPT* in neurons would further stabilize the microtubular system, leading to conserved intracellular/axonal distribution of metabolically important molecules under the conditions of reduced cholesterol availability. It should be noted that this differential effect of statins on *MAPT* expression in astrocytes and neurons would also likely change the relative proportion of astrocyte- to neuron-derived tau that reaches CSF.

The differences between simvastatin and pravastatin on gene expression were not only present in terms of the extent of the relative effect, but in some instances they had a different pattern of action in target cells. For example, under the employed incubation conditions, simvastatin affected expression of *GSK3β* and *CDK5* in neuronal cells, while pravastatin did not. In contrast, pravastatin had an effect on expression of neuronal *APP*, while simvastatin

did not. These findings suggest that these two statins could have significantly different effects on target cells.

Statins are among the most widely used medications in developed countries, and their use has been correlated with a significant reduction not only in cardiovascular disease and death rates, but also in reduction of numerous other diseases and conditions, including diseases of the central nervous system. For example, population studies suggested that statin users have significantly lower risk of dementia, including AD (2, 3), and clinical studies suggested that use of statins may also improve other neurological conditions, such as multiple sclerosis, Parkinson's disease, and traumatic brain injury (43–47). However, some studies of effects of statins on the brain and brain cells suggested that statins have potential not only to promote health but also to induce harm in the central nervous system. For example, a recent study by Coetsee et al. (48) has shown that statins induce significant DNA damage in neuronal cells in vivo. Other studies reported activation of both pro- and anti-inflammatory pathways, increased cell death, and higher susceptibility to oxidative damage in the brain tissue or brain cells exposed to statins (49–53). These controversial reports on both beneficial and harmful effects of statins in vitro and in vivo suggest that, despite their widespread use, statin effects on the brain need to be better understood. Data from our study support a concept that the use of statins needs to be evaluated on an individual basis and carefully monitored for potentially detrimental effects of statins on the brain.

In summary, our study has shown that effects of statins on expression of genes involved in neurodegenerative processes are statin and cell dependent. Furthermore, our data suggest a potential mechanism involved in development of statin-induced cognitive impairment in vulnerable patients that requires further study. 

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