

Plasma 24S Hydroxycholesterol Response to Statins in Alzheimer's Disease Patients: Effects of Gender, CYP46, and ApoE Polymorphisms

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Published online: 22 June 2007
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Abstract A number of epidemiologic studies suggest an association between plasma total cholesterol and risk for Alzheimer's disease (AD). Additionally, it has been suggested that treatment with statins, drugs that block cholesterol biosynthesis, lower the incidence and prevalence of AD and of vascular dementia. This review provides an overview of cholesterol transport within the central nervous system and the impact of statins on brain cholesterol metabolism in subjects with AD. Brain cholesterol is converted to 24-S-hydroxycholesterol, a reaction catalyzed by CYP46. The oxysterol traverses the blood–brain barrier and is transported to the liver by plasma lipoproteins. The levels of 24-S-hydroxy-cholesterol are a reflection of brain cholesterol turnover. Subjects with AD reportedly have high levels of the oxysterol possibly reflecting neuronal death with release of cell membrane cholesterol. We show gender dimorphism in plasma levels of 24-S-hydroxycholesterol in subjects with AD and significant reductions in plasma levels of the oxysterol during treatment with standard doses of statins (lovastatin, simvastatin, and pravastatin). Polymorphisms of apolipoprotein E and CYP46 do not influence the effect of statins on plasma levels of 24-S-hydroxycholesterol. There were

no untoward effects of the standard doses of statin for the duration of treatment. Statins are currently in trial to determine their effect on the course of AD.

Keywords Cholesterol metabolism · Alzheimer's disease · Hydroxycholesterol

Introduction

Both direct and indirect evidence link cholesterol metabolism and Alzheimer's disease (AD). Although cholesterol accounts for approximately 23 mg/g of brain weight, the synthetic rate of brain cholesterol in adults is very low (Dietschy and Turley 2004). The rate-limiting enzyme for brain synthesis of cholesterol is 3-hydroxy-3-methylglutaryl coenzyme A reductase, which can be regulated by plasma lipoproteins, as shown in primary glial cell cultures (Shah and Johnson 1988) and can be inhibited competitively by statins (Lennernas and Fager 1997). Because the adult brain synthesizes little cholesterol and acquires very little from plasma, it must recycle its cholesterol within the central nervous system (CNS) and must also have its own cholesterol-transporting lipoprotein system, as cholesterol has limited water solubility. Cholesterol catabolism in brain involves conversion to the oxysterol 24S-hydroxycholesterol by the oxidative enzyme CYP46 (Bjorkhem et al. 1997). The oxysterol, which is more hydrophilic than cholesterol, traverses the blood–brain barrier and is largely transported in plasma by low-density lipoprotein (LDL) for excretion by the liver. Because more than 90% of plasma 24S-hydroxycholesterol is from brain (Bjorkhem et al. 1998), plasma levels of the oxysterol may reflect brain cholesterol turnover. However, levels of 24S-hydroxycholesterol are strongly influenced by the concentration of circulating lipoproteins.

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proteins and the rate of hepatic clearance (Bjorkhem et al. 1998).

AD is thought to be related to abnormal processing of the cell membrane-associated amyloid precursor protein, forming a soluble beta amyloid molecule that self-aggregates and is deposited as extracellular amyloid plaques in brain tissue (Walsh and Selkoe 2004). Amyloid plaques contain the lipid transporter apoE, and apoEε4 is a known risk factor for AD (Weiner et al. 1999). Elevated levels of 24S-hydroxycholesterol have been found in AD patients' plasma (Papassotiropoulos et al. 2000; Lutjohann et al. 2000), possibly as a reflection of neuronal death with release of cell membrane cholesterol. In addition, depletion of cholesterol inhibits beta amyloid production in vitro (Simons et al. 1998). Because some epidemiologic studies indicated that treatment of dyslipidemia with statins appeared to lower the prevalence and incidence of AD and of vascular dementia (Wolozin et al. 2000; Jick et al. 2000) and because very high doses of simvastatin reduced levels of 24S-hydroxycholesterol in patients with AD (Simons et al. 2002), it was of interest to determine if levels of 24S-hydroxycholesterol could be reduced in subjects with AD by treatment with conventional doses of statins and other hypolipidemic agents and if polymorphisms for apoE and CYP46 influence the effect of statin treatment on plasma oxysterol level. This review summarizes the findings previously published elsewhere (Weiner et al. 1999; Vega et al. 2003; Vega et al. 2004). First, a brief overview of brain cholesterol metabolism is provided.

Cholesterol Transport in the Brain

Cholesterol comprises 22% of the gray matter, 27.5% of the white matter, and 27.7% of myelin (Morell et al. 1994). Cholesterol exists in three forms within cells: esterified, non-esterified, and oxysterols. Non-esterified cholesterol is mostly found in membranes; esterified cholesterol is stored in droplets of discrete volume. In the brain, this pool of cholesterol is recycled between cells and extracellular compartments via lipoproteins. Cholesterol derived from the intracellular pools also can be converted into oxysterols. The latter comprises a very small pool (10^{-3} to 10^{-6} the amount of intracellular cholesterol).

Intracellular cholesterol levels are regulated by sterol entry through lipoprotein receptors and efflux mediated by ABCA1 and apoE (Fig. 1). There are at least two intracellular sterol sensors, a nuclear receptor LXR (liver X receptor) and SREBP (sterol-regulating element-binding protein). SREBP, activated by free cholesterol, induces gene transcription that modulates LDL-receptor number on the surface of cells and de novo synthesis of cholesterol by HMGCoA reductase. LXR, a nuclear receptor, is activated by a key brain oxysterol, 24-S-hydroxycholesterol, which

induces LXR regulation of ABCA1 and apoE gene transcription. ABCA1 is a sterol transporter located on the surface of cells. It facilitates transfer of intracellular free cholesterol to extracellular lipoproteins containing apoA-I (LpA-I). These lipoproteins have a high density in a salt gradient; for this reason, they are also known as high-density lipoprotein (HDL; Fig. 1). In turn, the cholesterol acquired by LpA-I is esterified by lecithin cholesterol acyltransferase (LCAT) aided by cofactor apoA-I, leading to the production of a “mature” LpA-I lipoprotein. LpA-I transports cholesterol in the CNS, and it may also shuttle across the blood–brain barrier. LpE is a lipoprotein of high density that is produced within the CNS, and it can exchange cholesterol esters with LpA-I. LpE transports cholesteryl esters. The intracellular cholesterol ester pool is in equilibrium with free cholesterol, which is esterified by a brain-specific acyl-CoA cholesterol acyltransferase (not shown in Fig. 1).

Very little is known about the physical–chemical properties, specific function, and metabolism of the CNS lipoproteins in healthy subjects and in those with AD. We have studied lipid transport factors in plasma and cerebrospinal fluid (CSF) of AD patients and age- and gender-matched controls (Table 1). Levels of plasma and CSF apo E, apo A-I, apo A-II, apo B, were quantified immunochemically. There was no relationship between plasma and CSF concentrations of apoE and no difference between AD patients and controls (Table 1). There was no correlation between plasma and CSF apoA-I levels. ApoA-I concentration was similar in plasma for AD patients and controls,

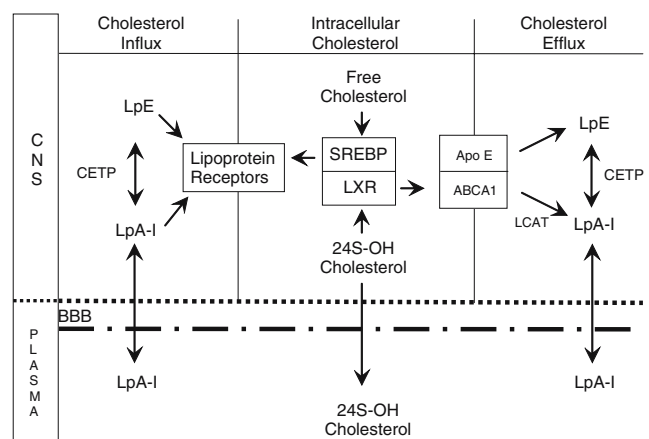


Figure 1 Hypothetical scheme of cholesterol balance in the CNS. Intracellular cholesterol balance is maintained by a “free cholesterol sensor” SREBP, and an oxysterol sensor, LXR. These “sensors” induce gene transcription for proteins that regulate sterol entry into the cells by lipoprotein receptors and sterol efflux mediated by ABCA1 and apo E. A key enzyme also regulated by LXR is CYP46 that catalyses the conversion of cholesterol to 24S-hydroxycholesterol, the main oxysterol derived from brain cholesterol. Drugs that are potent activators of LXR also induce β -amyloid protein secretion

but higher in AD CSF (Table 1). Molar ratios of apoE to apo A-I in plasma and CSF did not differ between AD patients and controls (Table 1). Levels of LCAT and CETP were similar in plasma and CSF of AD patients and controls (Table 2); however, the activity of these proteins was much lower in CSF than in plasma (Table 2). There was a significant correlation between apoE concentration and LCAT activity in plasma and CSF and between CSF apoA-I concentration and CSF LCAT activity. ApoA-II and apoB were not detected in CSF. There were no significant correlations between plasma levels of apoA-I or apoE and their counterparts in CSF.

We suggest that CSF lipoproteins differ markedly from plasma lipoproteins in apolipoprotein composition and function (Fig. 1). The main CSF lipoproteins appear to contain apoE and A-I. In contrast to plasma, there are no lipoproteins containing apo B in the CSF of AD or control subjects. The data suggest that neither LpA-I or LpE is derived from plasma, but this remains to be proven with certainty. Additionally, AD patients seem to have higher levels of CSF apoA-I than control subjects. This observation raises the question of whether AD subjects have a greater need for “shuttling” cholesterol within the CSF.

Effect of Statins on 24-S-Hydroxycholesterol in AD

There is growing interest in determining whether statins reduce risk for AD or slow the progression of the disease. Several statins are now approved by the Food and Drug Administration for treatment of hypercholesterolemia and patients with established coronary heart disease (CHD). The statins are lovastatin, simvastatin, pravastatin, atorvastatin, and rosuvastatin. Atorvastatin and rosuvastatin are synthetic drugs that are the most potent competitive inhibitors of HMGCoA reductase. Lovastatin, simvastatin, and pravastatin were employed in multicenter trials that

Table 2 Plasma and CSF activity of cholesterol ester transfer protein and lecithin cholesteryl acyl transferase^a

		Plasma	Cerebrospinal fluid	
		Activity	Activity	Percentage of plasma
CETP (% transfer)	Patients	32.7±2.2	1.27±0.03 ^b	4.08±0.31
	Controls	35.3±2.4	1.19±0.03	3.56±0.34
LCAT (nmol h ⁻¹ ml ⁻¹)	Patients	322±61	5.2±1.1 ^b	1.70±0.16
	Controls	331±66	4.7±1.6	1.49±0.16

^a Mean±SEM

^b Not significantly different from controls

showed relative efficacy and safety and risk reduction from CHD. In the few trials involving AD, high doses of simvastatin have been employed. It is of interest to determine whether standard doses may be as effective in treatment of AD. We conducted a study that compared several statins for relative safety and efficacy in lowering plasma levels of 24S-hydroxycholesterol in AD (Vega et al. 2003). Study subjects met accepted criteria for probable AD (McKhann et al. 1984). None had a history of cardiovascular disease, and none were taking hypolipidemic agents or had plasma total cholesterol <160 mg/dl. Subjects were randomly assigned to 6 weeks of treatment with various hypolipidemic agents; 40 mg/day of lovastatin, simvastatin, or pravastatin or 1 gm/day of extended release nicotinic acid. Lovastatin and simvastatin cross the blood-brain barrier, while pravastatin does not (Saheki et al. 1994). Biochemical determinations were made while subjects were fasting during pre-treatment and after 6 weeks as described elsewhere (Vega et al. 2003).

There were 61 subjects enrolled. In the 44 subjects who completed the hypolipidemic agent study, all three statins reduced levels of plasma lathosterol, 24S-hydroxy-cholesterol, LDL-cholesterol, and total cholesterol. Figure 2 shows the changes observed during simvastatin therapy. This pattern was similar for subjects taking pravastatin and lovastatin. The lathosterol-to-campesterol and 24S-hydroxycholesterol-to-LDL-cholesterol ratios were reduced significantly by all three statins, but the ratio of 24S-hydroxycholesterol-to-total cholesterol was unchanged. Extended release nicotinic acid reduced 24S-hydroxycholesterol by 10% and LDL-cholesterol by 18.1%. None of the agents lowered plasma concentration of Apo E. The lathosterol-to-campesterol ratios were reduced by 46.2% for all patients treated with statins; 24S-hydroxycholesterol-to-LDL-cholesterol ratios were increased by 128.6%, and the ratios of 24S-hydroxycholesterol-to total cholesterol remained unchanged. Extended release nicotinic acid had no significant effect on the ratios of the sterols.

Table 1 CSF and plasma levels of apolipoproteins in AD patients and controls

		Plasma	Cerebrospinal fluid
		(mg/dl±SEM)	
Apo E	Patients	4.2±0.4	0.39±0.02 ^b
	Controls	4.8±0.3	0.36±0.03 ^b
Apo A-I	Patients	129±4	1.08±0.07 ^{a,b}
	Controls	135±5	0.88±0.05 ^b
Apo E/A-I (molar ratios)	Patients	0.03±0.03	0.30±0.02 ^b
	Controls	0.03±0.02	0.35±0.03 ^b

^a Significantly different from CSF of controls; $p=0.03$

^b Significantly different from plasma levels; $p<0.01$

Data obtained in 11 AD patients and 14 controls of similar age and BMI. AD age of onset ranged from 63 to 78 years.

CYP46 and apoE genotyping was available for 53 subjects; 39 of these completed 6 weeks of treatment (Ingelsson et al. 2004). Baseline levels of 24S-hydroxycholesterol were significantly higher in women than men (Fig. 2a), and the ratios of 24S hydroxycholesterol-to-LDL cholesterol were higher in women than in men (Fig. 2b). Levels of HDL cholesterol also were significantly different (59 ± 18 vs 45 ± 9 ng/dl) and as was the total cholesterol (220 ± 5 vs 200 ± 7 mg/dl). There was no association between polymorphisms of CYP46 or apoE with plasma levels of 24S-hydroxycholesterol or with response to statin treatment (Vega et al. 2004). Women had similar percentage reduction in 24S-hydroxycholesterol as did men. All statins employed had similar effects on lathosterol, LDL cholesterol, and 24S hydroxycholesterol levels (Fig. 3).

We were not able to determine from our studies whether the reduction by hypolipidemic agents in plasma 24S-hydroxycholesterol was due primarily to reduced brain cholesterol synthesis or reduced LDL concentration.

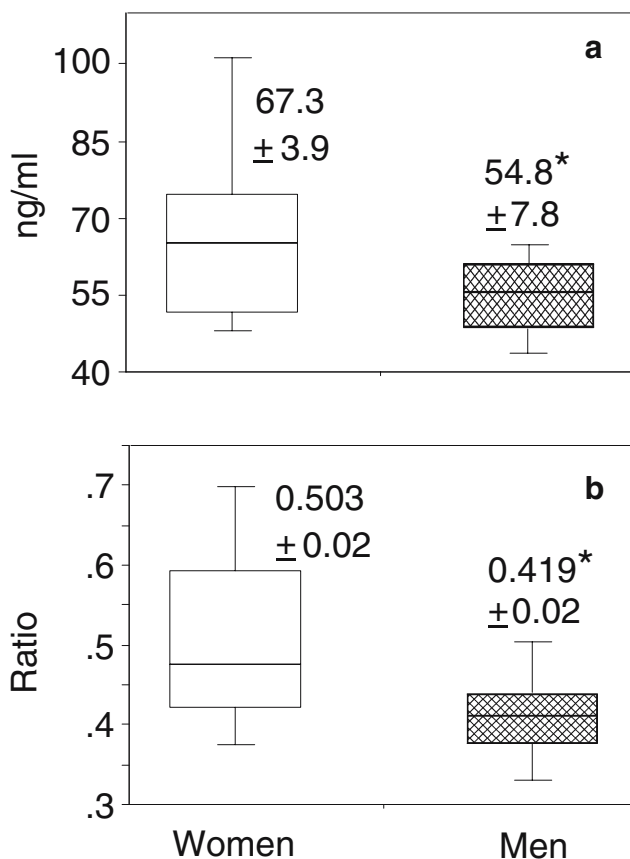


Figure 2 Levels of plasma 24S hydroxycholesterol (ng/ml) (a) and ratios of 24 S hydroxycholesterol-to-LDL-cholesterol in AD subjects (b). Gender differences are significantly different ($*P < 0.05$)

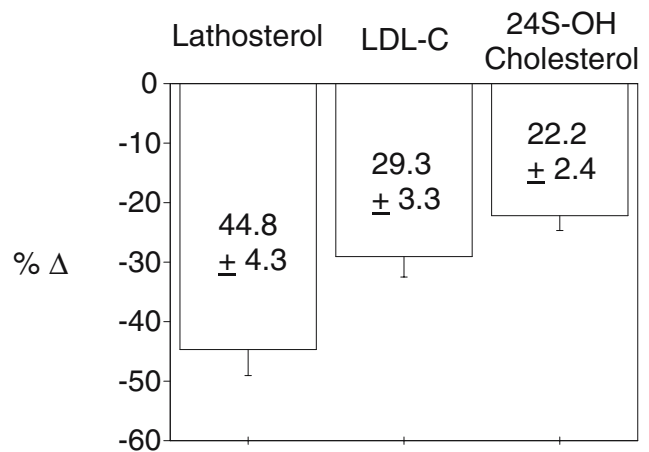


Figure 3 Effect of standard doses of simvastatin (40 mg/day) on plasma levels of lathosterol, LDL-cholesterol, and 24 S-hydroxycholesterol. Data are expressed as percent change (Δ) from baseline levels \pm SE

Lathosterol/campesterol ratios were calculated because campesterol absorption is not changed by statins. Therefore, the lathosterol-to-campesterol ratio provides an index of cholesterol biosynthesis. Cholesterol synthesis appears to have been reduced because the ratio was reduced by statins, but the ratio probably reflects reduced hepatic, not brain cholesterol synthesis. More work is required to determine the mechanism of 24S-hydroxycholesterol reduction by statins. Furthermore, the putative effect of statins on AD may be unrelated to their hypolipidemic properties. For example, simvastatin and lovastatin reduce levels of intracellular and extracellular beta amyloid in primary cultures of hippocampal neurons and mixed cortical neurons (Fassbender et al. 2001); guinea pigs treated with high doses of simvastatin showed reduced A β 42 and A β 40 CSF and brain homogenate levels (Fassbender et al. 2001). Controlled-release lovastatin also decreases beta-amyloid peptide in patients with hypercholesterolemia (Friedhoff et al. 2001). Studies are in progress to determine if long-term statin treatment will slow AD progression. One preliminary study (Sparks et al. 2005) suggests that this might be the case. The possibility that a polymorphism in the CYP46 gene (intron 2 AA) might have a relationship to AD has been proposed (Papassotiropoulos et al. 2003), but has recently been called into question (Ingelsson et al. 2004).

Acknowledgment This work was partially supported by a grant from the Wallace, Barbara and Kelly King Charitable Trust, a Veterans Affairs Medical Center Merit Review Grant, the Moss Heart Foundation, NIA Grant P-30-AG12300 and the UT Southwestern General Clinical Research Center Grant (NIH # M01-RR00633). Several collaborators have contributed to this work, including Drs. K. von Bergman, D. Lutjohan, F. Tato, S. Speciale, and H. Kölsch.

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