


Linking lipids, Alzheimer's and LXRs?

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Deposition of the β -amyloid ($A\beta$) peptide is thought to underlie development of Alzheimer's disease (AD). This pathological linkage has spurred considerable interest in therapeutic strategies to reduce $A\beta$ production. It is becoming increasingly clear that altered cholesterol homeostasis can modulate $A\beta$ production and/or accumulation. In this review, we discuss the molecular pathology of AD, the cholesterol connection and recent data suggesting that the oxysterol receptor, liver X receptor LXR (NR1H2 and NR1H3), may modulate these events.

Received February 6th, 2004; Accepted March 3rd, 2004; Published April 5th, 2004 | **Abbreviations:** $A\beta$: β -amyloid peptide, amyloidogenic peptide product of γ -secretase cleavage of CTF; **ABCA1**: ATP-binding cassette protein lipid transporter; **AD**: Alzheimer's disease; **apoE**: apolipoprotein E; **APP**: Amyloid precursor protein; **CTF**: Membrane-bound C-terminal fragment of APP generated by an α -secretase or β -secretase cleavage event; **LXR**: liver X receptor. | Copyright © 2004, Patel and Forman. This is an open-access article distributed under the terms of the Creative Commons Non-Commercial Attribution License, which permits unrestricted non-commercial use, distribution and reproduction in any medium, provided the original work is properly cited.

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Epidemiology of Alzheimer's disease

Alzheimer's disease (AD) is characterized by a progressive, age-related cognitive decline that ultimately results in memory loss, loss of language skills, difficulty performing routine tasks, disorientation and personality changes. Approximately 4.5 million people in the US currently suffer from AD and the incidence has been estimated at ~20% for individuals >75 years of age [Hebert et al., 2003]. The outlook is bleak in our aging population: disease prevalence doubles every 5 years over age of 65 and the number of affected individuals is expected to reach 13 million by 2050 [Hebert et al., 2003]. This estimate is a conservative one, since metabolic disease may increase the risk of AD, and hypercholesterolemia, diabetes and obesity are at epidemic levels. Anti-cholinesterases are currently used to limit the progression of AD but their effectiveness is far from ideal. There is thus a critical need to understand the molecular events that underlie AD and to translate this information into more effective therapies.

Molecular pathology of AD

The two pathological hallmarks of AD are the accumulation of intracellular neurofibrillary tangles and extracellular amyloid plaques (also known as senile or neuritic plaques). The major constituent of the amyloid plaque is a peptide known as $A\beta$ or β -amyloid [Masters et al., 1985; Wong et al., 1985] and aggregation of this peptide is now accepted as a causative factor in the disease [Hardy and Selkoe, 2002]. The $A\beta$ peptide arises via cleavage of the amyloid precursor protein (APP), a glycosylated, single transmembrane protein that is a substrate for several membrane bound proteases (Figure 1). APP is first cleaved by either α - or β -secretase. This cleavage occurs in the extra-cellular or luminal space [Kinoshita et al., 2003; Kojro et al., 2001] to yield a 105-125 kDa secreted N-terminal fragment ($APP\alpha$ or $APP\beta$), and the membrane-bound C-terminal fragment (CTF). The CTF is subsequently cleaved in the golgi-endosomal network by γ -secretase [Pasternak et al., 2003; Siman and Velji, 2003]. This protease cleaves

the CTF within the intra-membrane domain between 37-43 amino acids C-terminal to the β -secretase site. The γ -secretase products are referred to as $A\beta$ (β -CTF) or $p3$ (α -CTF) as they are derived from cleavage by β/γ - or α/γ -secretases, respectively. The most common β/γ -secretase cleaved product is $A\beta_{40}$ (85-95%), with $A\beta_{42}$ (5-15%) being the second most abundant product. Note that the initial "choice" between α - and β -secretase has dramatic consequences for AD: cleavage by β/γ -secretase results in $A\beta$ production whereas α -secretase cleaves within the $A\beta$ sequence. Thus, α -secretase not only precludes formation of $A\beta$ but results in production of the non-amyloidogenic $p3$ peptide. It should be noted that the sequence of the mouse $A\beta$ peptide diverges slightly from that of humans and has less predilection to aggregate into plaques. As a result, murine models of AD were developed by transgenic expression of human APP with familial mutations.

Cholesterol and Alzheimer's

The role of cholesterol in the pathogenesis of AD came to the forefront in 2000, when two groups independently reported that subjects that were treated with 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors – the statins – had a significantly lower prevalence of AD. In both studies, the statins decreased AD outcome by about 70% [Jick et al., 2000; Wolozin et al., 2000]. The statins act by inhibiting synthesis of mevalonic acid, a rate-limiting metabolite produced early in the cholesterol biosynthetic pathway. These findings suggest that elevated levels of cholesterol or some other biologically active precursor (e.g. farnesylpyrophosphate, geranylgeranylpyrophosphate) is associated with the development of AD. In support of a specific connection to cholesterol, four studies have reported an association between mild hypercholesterolemia and risk for AD. In earlier work, Sparks et al. [Sparks et al., 1993] had found that subjects who died of atherosclerotic heart disease, which is associated with elevated total cholesterol, had significantly greater numbers of amyloid plaques

compared to age-matched controls without atherosclerotic disease.

The cholesterol-statin-AD link has also been observed in experimental settings. Fassbender et al. [Fassbender et al., 2001] showed that guinea pigs fed large doses of simvastatin for as little as three weeks had 50% lower brain A β levels compared to the controls. In addition, high-cholesterol diets resulted in elevated intraneuronal A β levels in rabbits (A β sequence identical to humans), and increased plaque formation in transgenic mice expressing human APP [Refolo et al., 2000; Sparks, 1996].

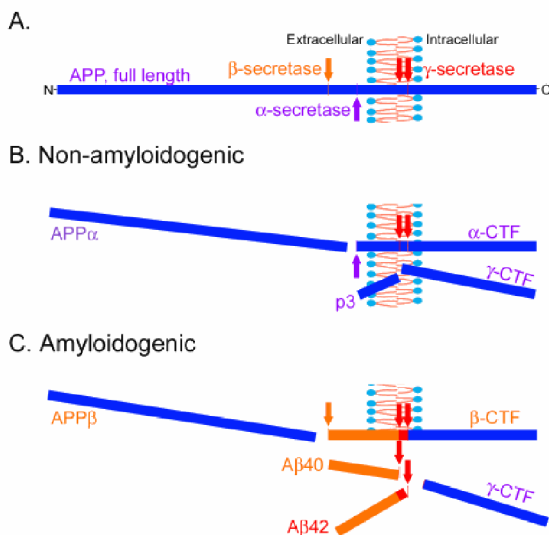


Figure 1. Proteolytic processing of A β -Precursor Protein (APP) by α -, β -, and γ -secretase. (A) The single-transmembrane domain-containing full-length APP is shown along with the cleavage sites for α -, β -, and γ -secretase. (B) Non-amyloidogenic processing of APP. α -secretase cleaves APP in the extracellular space to yield the N-terminal secreted APP α fragment and the transmembrane domain-containing α -CTF. The α -CTF is then cleaved by the γ -secretase to produce the non-amyloidogenic p3 and γ -CTF fragments. (C) Amyloidogenic processing of APP. APP is cleaved by β -secretase in the extracellular domain to produce APP β and β -CTF. The γ -secretase can cleave β -CTF at position 40 or 42 amino-acids C-terminal to the β -secretase site. Thus, the β/γ -secretase cleavage yields the amyloidogenic A β 40 (orange) and A β 42 peptides (orange + red), and γ -CTF.

If AD arises via an increase in A β production/accumulation, how do alterations in cholesterol homeostasis affect the development of this disease? The answer may lie in the fact that APP/A β processing occurs within cholesterol-rich membrane domains, i.e. APP and the β - and γ -secretases are localized in cholesterol-rich lipid rafts [Bouillot et al., 1996; Eehalt et al., 2003; Marlow et al., 2003; Riddell et al., 2001; Wahrle et al., 2002].

Cholesterol homeostasis in the brain

Much like the liver, the brain expresses the machinery required for cholesterol uptake, intracellular trafficking, de novo synthesis and metabolic degradation. In the periphery, apolipoprotein AI (apoAI) and apolipoprotein E (apoE) serve as the major extracellular cholesterol acceptors. ApoE is a ligand for all members of the LDL receptor family, and directly mediates hepatic uptake of

very low-density lipoprotein (VLDL). In the brain, extracellular cholesterol is transported via lipoprotein-like particles that contain astroglia- and microglia-derived apoE and apoJ [Danik et al., 1999]. The apoE-containing particles are endocytosed by neuronal lipoprotein receptor related protein (LRP) [Williams et al., 1998]. As in the periphery, these lipoprotein complexes are transported to lysosomes through endosomal pathways and ultimately other compartments via the Neiman-Pick C1 protein (NPC1) [Runz et al., 2002; Zhang et al., 2001].

It is important to note that lipoproteins cannot pass the blood-brain barrier. This has two critical implications. First, peripheral and central cholesterol pools are not readily interchangeable and are largely maintained as separate pools [Koch et al., 2001]. Second, the brain cannot rely on peripherally-derived cholesterol to satisfy its massive cholesterol requirements. Instead, the brain produces its own cholesterol via the standard HMG-CoA reductase-limited biosynthetic pathway. As in the liver, excess cholesterol is removed via catabolism to more polar degradation products: the liver utilizes CYP7A1 (cholesterol 7 α -hydroxylase) to metabolize cholesterol to bile acids whereas the brain predominantly utilizes CYP46 (cholesterol 24-hydroxylase) to convert cholesterol to 24(S)-hydroxycholesterol [Lund et al., 2003]. This 24-hydroxylated metabolite can exit via the blood-brain barrier where it is further metabolized and ultimately eliminated by the liver.

APP/A β processing and cholesterol trafficking

Although total cholesterol levels are tightly regulated in the brain, the precise location of individual cholesterol molecules can be highly dynamic due to endosomal uptake pathways and NPC1-mediated intracellular trafficking. Moreover, the concentration of cholesterol in different intracellular membranes is not uniform: cholesterol content increases successively from the endoplasmic reticulum to the plasma membrane, and within the plasma membrane, cholesterol is organized into distinct structural pools that include lipid rafts and caveolae. Presence of cholesterol within the membrane alters the fluidity of the surrounding membranes and impacts biological events that occur within those membranes [Schroeder et al., 2001]. Thus, dysregulation of cholesterol trafficking could contribute to AD as β/γ -secretase dependent APP/A β processing occurs within cholesterol-rich lipid rafts and endosomes.

Several lines of evidence support the above hypothesis. Intracellular cholesterol trafficking can be altered by expressing an NPC1 mutant. This leads to the accumulation of cholesterol in late endosomes and lysosomes. Mice expressing the mutated NPC1 show no changes in amounts of α - or β -secretase activity but exhibit increased γ -secretase activity and accumulate A β 40 and A β 42. Increased activity occurs without any detectable change in the amount of the PS1 subunit of γ -secretase. This suggests that changes in membrane

cholesterol distribution stimulates γ -secretase activity and ultimately A β production [Burns et al., 2003]. Similarly, treatment of neurons with drugs that block cholesterol trafficking from lysosomes (U18666A, imipramine) also increased γ -secretase activity [Runz et al., 2002]. Enhanced γ -secretase activity was associated with abnormal localization of γ -secretase subunits (PS1 and PS2) in cholesterol sorting vesicular compartments. Thus, changes in membrane cholesterol trafficking appear to alter the activity of γ -secretase, a membrane bound APP processing enzyme.

Treatment of cells with statins and cholesterol-depleting agents suggest that cholesterol may also affect the other APP processing enzymes, i.e. α - and β -secretase. Specifically, decreasing cholesterol is associated with a rise in the non-amyloidogenic activity of α -secretase and a decrease in the amyloidogenic β -secretase activity [Fassbender et al., 2001; Kojro et al., 2001;]. The net effect of these changes are consistent with the AD-promoting effects of cholesterol. The differential effects on α - and β -secretase may be related to the fact that α -secretase is primarily active at the cell-surface, while β -secretase (BACE) is associated with lipid rafts [Kojro et al., 2001; Riddell et al., 2001]. This distinct localization of the α - and β -secretase also suggests that α -cleavage of APP could be accentuated if APP is retained longer on the cell surface. In agreement, Kojro et al. [Kojro et al., 2001] found that depletion of membrane cholesterol increased membrane fluidity, decreased endocytosis, and was associated with increased α -cleavage and less A β .

Other interventions that alter cellular cholesterol transport have also been shown to alter APP cleavage. For example, overexpression of the ABCA1 lipid transporter reduced A β secretion in Neuro2a cells (murine neuroblastoma) expressing human APP with a swedish familial mutation (APP^{sw}, Figure 1) [Sun et al., 2003]. Although ABCA1 can promote cholesterol efflux from macrophages, little cholesterol efflux was observed in these Neuro2a cells. It has been suggested that ABCA1 is primarily a phospholipid transporter; its ability to promote cholesterol efflux may be secondary to an ABCA1-mediated redistribution of cholesterol from inner to outer membrane surfaces [Vaughan and Oram, 2003] followed by the binding of the outer membrane cholesterol to extracellular acceptor proteins (e.g. apoA1 and apoE). In adipocytes, ABCA1 has been shown to shift cholesterol from membranes to lipid droplets [Le Lay et al., 2003]. Thus, the ABCA1-dependent reduction of A β may be related with cholesterol redistribution rather than efflux. When viewed as a whole, this body of literature suggests that intracellular cholesterol depletion and/or redistribution has important consequences for the proteolytic processing of APP and the production of A β .

LXR and cholesterol homeostasis

There are a number of interesting links between the Liver X Receptors (LXR α /NR1H3 and LXR β /NR1H2) and the above biology. LXR α was originally identified as an orphan receptor highly expressed in hepatic tissue. Subsequent studies demonstrated that LXRs are central

players in cholesterol homeostasis throughout the periphery (for detailed reviews see [Joseph and Tontonoz, 2003; Joseph and Tontonoz, 2003]. Briefly, LXR α is abundant in tissues that metabolize cholesterol including liver, intestine and macrophages ; LXR β is ubiquitously expressed. Both receptors bind to and are activated by cholesterol derived oxysterol ligands. When activated by ligand, LXRs induce CYP7A-1 mediated cholesterol degradation in the liver (rodents) and promote cholesterol efflux from macrophages, hepatocytes and intestinal enterocytes. Enhanced efflux is mediated by LXR-dependent activation of cholesterol/lipid transporters (ABCA1, ABCG1, ABCG5 and ABCG8) and cholesterol acceptor proteins (apoE). These effects are biologically significant as synthetic LXR agonists increase plasma HDL cholesterol, stimulate cholesterol excretion into the bile and the feces, decrease hepatic cholesterol content and can reduce atherosclerotic lesions by ~50% in various murine models [Cao et al., 2002; Joseph et al., 2002; Plosch et al., 2002; Schultz et al., 2000; Yu et al., 2003].

So what about LXRs and the brain? Both subtypes are expressed in the CNS at significant levels: LXR α mRNA levels range from 7-29% of that in the liver, while LXR β levels are 2-5 fold higher in the brain than in liver. The levels of LXR α in cultured neurons and glia are 2% and 17% compared to the liver, and that for LXR β are 1.1- and 3.8-fold, respectively [Whitney et al., 2002]. Are these receptors functional? The answer is yes. Astrocytes treated with synthetic LXR ligands exhibit enhanced cholesterol efflux and increased expression of LXR target genes including ABCA1, ABCG1, and apoE [Fukumoto et al., 2002; Koldamova et al., 2003; Liang et al., 2004; Whitney et al., 2002]. Lxr α / β -null mice show a variety of CNS defects including lipid accumulation, astrocyte proliferation and disorganized myelin sheaths [Joseph et al., 2002]. These studies demonstrate that LXRs modulate cholesterol homeostasis in the CNS and are required for normal CNS function.

Is there a connection between LXRs and AD?

The discussion above suggests that modulators of intracellular cholesterol content and/or distribution can modulate APP processing. LXR clearly meets this criterion and offers other potentially intriguing links to AD. For example, polymorphisms in apoE, an LXR-regulated gene, is the strongest predictor of late-onset AD [Corder et al., 1993]. CYP46 (cholesterol-24-hydroxylase) polymorphisms have also been linked to AD [Kolsch et al., 2002; Papassotiropoulos et al., 2003] and expression of this enzyme is shifted from neurons to glia in AD patients [Bogdanovic et al., 2001]. CYP46 converts cholesterol into an oxysterol ligand (24(S)-hydroxycholesterol) that can bind and activate LXRs in cultured cells. Although it remains unclear whether 24(S)-hydroxycholesterol is the active ligand that is bound to LXRs in vivo, these intriguing coincidences provide additional impetus to explore an LXR – AD connection.

LXR agonists modulate APP/A β processing

Three recent publications demonstrate that LXRs can indeed modulate proteolytic-processing of APP, albeit with conflicting conclusions. The Rebeck lab [Fukumoto et al., 2002] demonstrated that LXR agonists (22(R)-hydroxycholesterol or T0901317) increased both ABCA1 expression and A β secretion in Neuro2A cells; exogenous cholesterol carrier was not added. The increase in A β was likely mediated via ABCA1 as siRNA targeting this transporter decreased A β production. Using two human neuroblastoma cell-lines stably expressing APP^{swe}, the Lazo lab [Koldamova et al., 2003] also observed the expected increase in ABCA1 expression, enhanced cholesterol efflux and subsequent decrease in total cellular cholesterol. However, in sharp contrast to the previous group, LXR agonist (22(R)-hydroxycholesterol) reduced A β production. In these experiments, addition of apoA1 or apoE to the culture medium promoted significant cholesterol efflux, which was associated with further reduction in A β . Tall's group also found that LXR agonist (T0901317) enhanced ABCA1 expression and decreased A β production in mouse Neuro2A cells expressing human APP^{swe} [Sun et al., 2003]. The reduction in A β was related to a decrease in β - and γ -secretase cleavage. Moreover, the decrease in A β could be mimicked by overexpression of wild-type ABCA1 but not by a mutant that was defective in cholesterol transport and apoA1 binding. However, ABCA1-mediated reduction in A β was observed under conditions where there was little or no cholesterol or phospholipid efflux (i.e. without apoA1 or apoE in the media). Thus unlike Rebeck's group, both Lazo and Tall's groups find that LXR inhibits A β by inducing ABCA1 activity. Some discussion remains as to whether the relevant activity of ABCA1 is intracellular cholesterol redistribution vs. lipid efflux. The reasons for the discrepancies among the 3 groups could be related to differences in cell lines (mouse vs. human; wild-type vs. APP^{swe}+) and/or other methodologies.

Where do we go from here?

The potential to treat AD with a nuclear receptor ligand is quite exciting. However, given the above discrepancies, it will be critical to determine the net effect of LXR agonists on APP processing in vivo. Experiments in cell culture will be valuable in sorting through mechanistic questions but given the interactions between neurons and glia, it will be important to look at LXR effects in an in vivo setting. This is complicated by the choice of animal model. As mentioned above, the wild-type mouse does not develop AD pathology as the mouse A β peptide does not aggregate. This particular problem has been overcome by expressing human APP variants in several transgenic mouse models. This is not a perfect solution for many reasons, not the least of which is that cholesterol homeostasis is differentially regulated in mouse and man. Indeed, this issue has come to the forefront before for LXR. LXR agonists can raise HDL-cholesterol in several mouse models. However, mice do not express cholesteryl

ester transfer protein (CETP) and the effects of LXR agonists on HDL-cholesterol are lost in transgenic mice expressing human CETP [Masson et al., 2004]. When it comes to cholesterol homeostasis and AD, these tales remind us to exercise more than the usual caution when extrapolating results to humans.

Another area of future interest is the relationship between peripheral and central cholesterol homeostasis and how LXR may contribute to each. As described above, it is believed that peripheral and central cholesterol pools are largely distinct. If this is the case, how does excess dietary cholesterol promote A β accumulation [Refolo et al., 2000]? Similarly, some of the statins that lower AD-risk are considered relatively impermeable to the blood-brain barrier [Wolozin et al., 2000]. These findings raise the possibility of important interactions between peripheral and central cholesterol homeostasis and that peripheral targets may be viable for anti-AD therapy. The effect of peripheral vs. central effects is particularly relevant to LXR as it can directly regulate cholesterol homeostasis in both compartments.

Summary

Cholesterol is proving to be an important factor in the development of AD. Population studies suggest that mild hypercholesterolemia can increase the risk of AD and decreasing cholesterol synthesis via statin administration may decrease the development of AD. Moreover, elevated cellular cholesterol content has been shown to favor production of the amyloid- β (A β) peptide, a pathognomic hallmark of AD. Genetic studies have suggested links between AD and several cholesterol control genes including the cholesterol acceptor ApoE (ϵ 4 polymorphism). LXR is expressed in the brain where it modulates cholesterol homeostasis and possibly APP processing. Many questions remain, but as a master regulator of cholesterol homeostasis, LXR can be considered a potential molecular target for the treatment of AD.

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