

Neurobiology of Lipids

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commentary

INDUCTION OF CHOLESTEROL EFFLUX IN THE CNS

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Intricate cellular mechanisms exist for maintaining proper cholesterol levels. Several recent studies of removal of excess cholesterol in the CNS have focused on the ABC-A1 lipid transporter and its regulation via the LXR nuclear hormone receptor. Cellular cholesterol is hydroxylated to form oxysterols (24-hydroxycholesterol in the brain), which bind LXR and promote gene transcription. LXR activation increases levels of ABC-A1 and apoE, which together act to remove cholesterol and other lipids from cells. Recent studies including Liang *et al.*¹ suggest manipulation of the LXR system alters brain cholesterol homeostasis and apoE levels, and thus could be beneficial in approaches to Alzheimer disease therapeutics.

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CHOLESTEROL HOMEOSTASIS AND ALZHEIMER'S DISEASE

The recent publication of Liang *et al.*¹ showing that LXR agonists induce apoE expression in astrocyte cells *in vitro* and in mouse brain *in vivo* continues a recent rise in interest in the area of CNS cholesterol efflux. Behind these studies are the connections

between cholesterol and Alzheimer's disease (AD), including work from over ten years ago on animal models² and genetic links with APOE.³ More recent epidemiology studies show high risk of AD associated with high cholesterol levels⁴ and low risk of AD associated with use of cholesterol lowering drugs.⁵ Studies of transgenic APP mice demonstrate that high cholesterol diets are associated with increased brain amyloid levels and cholesterol-lowering drugs lead to decreased amyloid levels.⁶

BRAIN CHOLESTEROL HOMEOSTASIS

One set of approaches to understanding the mechanisms for how high cholesterol affects AD risk and A β levels has involved *in vitro* systems: studying how cellular cholesterol levels alter A β production and clearance, cellular toxicity, and apoE levels (reviewed in Ref. 7). In these cellular paradigms, it is important to take into account that altering cellular cholesterol levels induces mechanisms to maintain cholesterol equilibrium, systems that are best understood in peripheral tissues, but which have important function in the CNS. For example, when cellular cholesterol levels are low there is induction of a number of genes via the transcription factor SREBP; these genes include HMG-CoA reductase for increased production of cholesterol and the low-density lipoprotein receptor (LDLr) for increased endocytosis of cholesterol-rich lipoproteins.⁸

There is another mechanism for induction of genes when cellular cholesterol levels are high, involving

a system for removal of excess cholesterol from cells via cholesterol efflux. Intracellular cholesterol is hydroxylated at specific sites by cytochrome P450 enzymes to produce oxysterols. CYP46 is a brain-specific enzyme that produces 24-hydroxycholesterol (24-OHch),⁹ which binds to the LXR nuclear hormone receptor (a commonly used synthetic agonist for LXR activation is TO-901317). LXR forms heterodimers with the RXR nuclear hormone receptor, which binds retinoic acid, and together these molecules promote transcription of genes containing LXR response elements.^{10,11} There are two isoforms of LXR, LXR α and LXR β ; LXR β is the predominant form found in the brain.^{12,13} A double knockout of LXR α and β causes dramatic accumulation of lipid-laden cells (particularly astrocytes) around the ventricles and the cerebrovasculature.¹⁴ Furthermore, these mice showed dramatic decreases in brain expression of many lipid metabolism genes, including LDLr and HMG-CoA reductase,¹⁴ demonstrating that LXR is important for normal brain cholesterol homeostasis.

Genes induced by LXR/RXR dimers include apoE and the ATP binding cassette (ABC) protein, ABC-A1. ABC-A1 is, in part, responsible for the transport of cholesterol and phospholipids from intracellular membranes to extracellular cholesterol binding molecules, such as apoE and apoAI.¹⁵ Mice treated with TO-901317 demonstrated increases of ABC-A1 and SREBP in the brain,¹³ but not CYP46 or LXR genes. Although not observed in the Whitney study,¹³ Liang *et al.* demonstrated that CNS apoE levels were increased after TO treatment for one week.¹ ApoE is induced in the brain after cellular damages,¹⁶ and is necessary for the removal of cellular debris.¹⁷ ABC-A1 is also induced after excitotoxic lesions in both glia and neurons,¹⁸ presumably also to remove lipids from damaged cells.

These *in vivo* studies are supported by a number of *in vitro* studies reporting that induction of LXR led to increases in ABC-A1 in neuronal and glial cells.¹⁸⁻²⁰ Similar treatments caused increases in apoE in astrocytoma cells.¹ In the presence of a lipid acceptor such as apoAI, these inductions can lead to increased cholesterol efflux from the cells, resulting in lower intracellular cholesterol levels.¹⁹ ApoE can induce cholesterol efflux from cells in culture, and this effect is isoform specific, with apoE2 the most efficient and apoE4 the least efficient.²¹ In cells secreting a cholesterol acceptor, such as glia expressing apoE, induction of cholesterol efflux by LXR agonists could occur in the absence of exogenous cholesterol acceptors.¹³ In the absence of such an acceptor, cholesterol

efflux is not increased,²⁰ although there is a redistribution of cholesterol to the plasma membrane.²²

CHOLESTEROL EFFLUX AND ALZHEIMER'S DISEASE

Three studies have focused on the effects of cholesterol efflux on metabolism of amyloid beta protein (A β), but with very different results. Our studies of several types of cells including primary neurons showed that compounds that induced cholesterol efflux increased secreted levels of A β 42 without changing processing of APP.¹⁸ We suggested that this effect might be due to increased efflux of intracellular A β . This hypothesis fits with the findings of Lam *et al.* showing that another ABC protein, p-glycoprotein, could act to transport A β across cell membranes.²³

Two other studies, in contrast, reported that induction of the LXR system resulted in reduction of total A β levels. Koldamova *et al.* found that induction of LXR caused a reduction in APP CTF's and more secreted APP, and that the changes were dependent on a reduction in cellular cholesterol levels.¹⁹ Sun *et al.* found that induction of LXR and overexpression of ABCA1 reduced A β and C99 levels, suggesting reduced β -cleavage of APP, but these effects occurred without a reduction in cellular cholesterol levels.²⁰ The sources of the different results of these three studies remain to be determined, but could result from the timing of the treatments and measurements, the differences in total cellular lipid levels, or the distribution of lipids among cellular membranes.

CHOLESTEROL EFFLUX AND AD THERAPEUTICS

Reducing cellular cholesterol levels by increasing cholesterol efflux is an attractive target for therapeutic intervention. Induction of cholesterol efflux by LXR agonists includes induction of apoE in many tissues,²⁴ including the brain.¹ ApoE may have both positive and negative effects on deposition of A β in transgenic mouse models of AD.²⁵ It is important to note that the APOE locus in humans is structurally altered from mice, with a duplication of the LXR binding element in humans²⁶; Liang *et al.* propose this difference could account for the relatively weak induction of apoE by TO in mouse brain compared to human astrocytoma cells.¹ For possible therapeutics, it will be important to consider the effects of inducing many genes including APOE, as well as long-term

changes to the cells after increasing cholesterol efflux. In order to understand chronic diseases like Alzheimer's disease, we cannot focus exclusively on acute changes in cellular metabolism of A β .

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