Review

Neurosteroids and Oxysterols as Potential Therapeutic Agents for Glaucoma and Alzheimer's Disease

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Abstract

Glaucoma is one of the most frequent causes of visual impairment worldwide and involves selective damage to retinal ganglion cells (RGCs) resulting in degeneration of neural pathways connecting retina to visual cortex. It is of interest that similarities in pathological changes have been described in Alzheimer's disease (AD), the most common cause of progressive memory loss and dementia in older people. Accumulation of amyloid-beta (Abeta) and hyperphosphorylated tau is thought to contribute to apoptotic neuronal death in Alzheimer's disease, and similar changes have been linked to apoptotic RGC death in glaucoma. Both glaucoma and Alzheimer's disease also suffer from a lack of effective treatments prompting a search for novel therapeutic interventions. Neurosteroids (NSs) (including oxysterols) are endogenous molecules synthesized in the nervous system from cholesterol that can modulate glutamate and GABA receptors, the primary mediators of fast excitatory and inhibitory neurotransmission in the brain, respectively. Because changes in the glutamate and GABA neurotransmitter systems contribute to the pathogenesis of AD and glaucoma, NSs are possible therapeutic targets for these disorders. In this review, we present recent evidence supporting pathological links between Alzheimer's disease and glaucoma, and focus on the possible role of NSs in these diseases and how NSs might be developed for therapeutic purposes.

Introduction

Despite its peripheral location, the retina is actually part of the central nervous system (CNS). Embryologically, the retina is derived from an out-pocketing of the neural tube, which is the precursor to the CNS including the brain and spinal cord [1]. Therefore, pathological processes and therapeutic strategies affecting the brain can be applicable to the retina [2]. Glaucoma is considered to be a neurodegenerative disease, and is one of the primary causes of visual impairment worldwide among older people [3,4]. Retinal ganglion cells (RGCs) transmit visual information from photoreceptor cells to the brain, and glaucoma is characterized by apoptotic death of RGCs [5]. Glaucoma and neurodegenerative disease such as Alzheimer's disease, the most common cause of progressive memory loss and dementia in older people, have several factors in common such as selective

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loss of neurons and deposition of amyloid-β peptide or highly soluble microtubule-associated protein (tau) [6,7]. Family history is also a significant risk factor for both diseases [8].

Neurosteroids including oxysterols are endogenously generated from cholesterol within the nervous system, and effectively modulate major neurotransmitter systems (particularly the glutamatergic and GABAergic systems) [9]. Although the precise pathological mechanisms have remained unclear, recent evidence indicates that neurosteroids may contribute to the pathogenesis of both glaucoma and Alzheimer's disease [10]. In this review, we will discuss the association of glaucoma and Alzheimer's disease, and focus on the possible role of neurosteroids in these illnesses, and how neurosteroids might be developed for therapeutic use.

Glaucoma and Alzheimer's Disease

Based on the results of retrospective chart review, Alzheimer's disease patients have a significantly increased incidence of glaucoma (24.5%) compared to controls (6.5%) [11]. A subsequent study from the same research group [12] found similar results with a prevalence of glaucoma in Alzheimer's disease patients and controls of 25.9% and 5.2%, respectively. Consistent with this, a cross sectional epidemiologic study revealed that the prevalence of primary open angle glaucoma (POAG), the most common type of glaucoma, was 23.8% in Alzheimer's disease patients compared to 9.9% in controls (p = 0.0002) [13].

Sartucci et al. [14] have shown an impairment of visual responses arising from the magnocellular pathway of visual processing using pattern electroretinograms and visual evoked potentials in patients with Alzheimer's disease, suggesting that the largest RGCs (M cells) are primarily affected in the illness. M-cells are more sensitive to contrast stimuli than other types of RGCs, and known to mediate specific visual functions for detecting quick motion. M-cells have larger receptive field, and make up approximately 5-10% of all RGCs.

Retinal involvement in early stage Alzheimer's disease is also suggested by results from imaging studies using optical coherence tomography (OCT). Parisi et al. [15] examined seventeen Alzheimer's disease patients and fourteen age-matched controls by OCT, and reported that in Alzheimer's disease patients, there was a significant reduction of nerve fiber layer (NFL) thickness compared to controls (59.5 ± 16.70 μm vs. 99.9 ± 8.95 μm, p<0.01). This morphological abnormality correlated with retinal dysfunction as revealed by abnormal pattern electroretinogram recordings [15]. A significant reduction of macular volume in Alzheimer's disease patients was also reported compared to controls using OCT (p<0.05), and the reduction in macular volume was related to the severity of cognitive impairment [16].

Conversely, the incidence of Alzheimer's disease in patients having glaucoma has also been investigated. Lin et al. [17] retrospectively analyzed a population-based cohort consisting of patients older than 60 years with POAG to investigate the risk developing Alzheimer's disease. After 8 years following a diagnosis of POAG, the prevalence rates of Alzheimer's disease were 2.85 (95% CI: 2.19-3.70) and 1.98 (95% CI: 1.68-2.31) per 1000 person-years in patients with and without POAG, respectively. Kaplan-Meier survival curves and the log-rank test revealed that POAG patients had an increased risk of developing Alzheimer's disease compared to controls without POAG (log-rank test, P=0.0189).

A population-based longitudinal cohort study (Three-City-Bordeaux-Alienor study) showed that participants with POAG have a four-fold increased risk of developing dementia (odds ratio=3.9, 95% confidence interval (CI) =1.5–10.4, p=0.0054) during a 3-year follow-up period [18]. An increased risk of dementia was also associated with 2 parameters of glaucoma progression (increase in vertical cup to disk ratio and decrease in rim to disk ratio). Recently, Lai et al. [19] calculated the odds ratio for developing Alzheimer's disease as 1.50 in subjects with glaucoma (95% CI=1.19-1.89) using a multivariable unconditional logistic regression model.

Other studies, however, have reported opposite results concerning subsequent risk of developing Alzheimer's disease in patients with glaucoma. Ou et al. [20] examined a nationally representative sample of persons in the U.S. with POAG newly diagnosed prior to 1994 and determined whether these persons have subsequent risk of developing Alzheimer's disease or other dementia (Alzheimer's disease/dementia) compared to a well-matched control population without glaucoma over a 14 year follow up period. Their analysis revealed that individuals older than 68 years diagnosed with POAG have a decreased risk of Alzheimer's disease compared to control

patients who were not diagnosed with POAG. In a nationwide case register study of patients with hospital admission or outpatient contact during the period from 1977 to 2001 in Denmark, the rate of subsequent Alzheimer's disease for patients with a diagnosis of POAG was compared with the rate for patients with primary angle-closure glaucoma (PACG), cataract, and osteoarthritis (OA) along with the rate for the general population [21]. This study reported that patients having POAG showed no increased risk of developing Alzheimer's disease compared with the other groups. Therefore, the association of glaucoma with Alzheimer's disease remains equivocal based on epidemiological data.

Histologically, Hinton et al. [22] showed remarkable decreases in optic nerve fiber density (from 50% to 33%) in 8 of 10 postmortem Alzheimer's disease patients compared with controls. The retinas in Alzheimer's disease patients showed disappearance of RGCs and appearance of reactive gliosis in the ganglion cell layer. These histological changes in the retina of Alzheimer's disease patients were markedly different from the findings in 10 age-matched controls. However, amyloid was not detected in the retinal and optic nerve. Blanks et al. [23] analyzed RGC density in 9 Alzheimer's disease patients, and found that a 43% decrease in RGC density occurred in the fovea. In the more peripheral region of the macula, RGC decreased by 24-26%. Sadun and Bassi [24] reported similar degeneration in the RGCs. Optic nerves from ten patients with Alzheimer's disease were histologically examined and compared with those from 5 age-matched controls. Morphometric analysis suggested that optic nerves in Alzheimer's disease showed predominant loss of the largest RGCs (M-cells). These results were consistent with results obtained using pattern electroretinograms and visual evoked potentials [14]. These results suggest that glaucoma and Alzheimer's disease likely share some common pathogenetic features.

In the next sections, we focus on the pathological roles of neurotoxic factors such as amyloid-β peptide and tau, both of which contribute to neuronal degeneration in Alzheimer's disease and glaucoma [25-27].

■ Amyloid-dependent mechanisms in glaucoma

Generation of neurotoxic amyloid-β peptide from sequential amyloid precursor protein (APP) proteolysis is a crucial step in the pathogenesis of Alzheimer's disease. APP is a transmembrane protein preferentially expressed in the brain, and metabolized by proteases including γ- and β-secretase complexes. Amyloid-β peptide is a polypeptide containing 37 to 49 amino acid residues, and its amyloid fibrillar form is the main component of the senile plaques that are characteristic of Alzheimer's disease lesions [28].

Both Alzheimer's disease and glaucoma are associated with apoptotic neuronal degeneration [29-31], and the activation of caspases is a central mechanism driving this cell death [29]. Caspase-3 induces abnormal APP processing and increases expression of amyloid-β peptide in RGCs [32] along with decreases in vitreous amyloid-β peptide levels (consistent with retinal amyloid-β peptide deposition) [33]. These findings suggest that APP exerts neuroprotection for RGCs, while APP fragments such as amyloid-β peptide are toxic in glaucoma. Presenilin 1 (PS1) and PS2 proteins play important roles in caspase-3-mediated abnormal APP processing. Most cases of early-onset familial Alzheimer's disease are caused by mutations in the genes encoding the PS1 and PS2 proteins, both of which undergo regulated endoproteolytic processing [34]. Consistent with this, Ning et al. [35] demonstrated significant age-dependent deposition of amyloid-β peptide in the retinal nerve fiber layer (NFL) and an age-dependent increase in TUNEL-positive RGC using two strains of bi-transgenic APP/presenilin 1 (PS1) mice. Amyloid-β peptide therefore may offer a novel therapeutic target in the treatment of glaucomatous neurodegeneration. An α2 adrenergic receptor agonist (brimonidine), used to lower intraocular pressure, has been recently reported to prevent RGC death through its effects on the amyloid-β peptide pathway (reduction of amyloid-β peptide) and soluble APPα (increase in APPα) in an *in vivo* rat glaucoma model [36].

Neuroinflammation is another possible contributor to the pathogenesis of Alzheimer's disease [37]. An increase in amyloid-β peptide deposition induces the activation of astrocytes as well as microglia [38]. Activated glia can secrete inflammatory chemical mediators and induce chronic inflammation. This inflammation is induced by amyloid-β peptide deposition, and accelerates to generate more amyloid-β peptide while weakening mechanisms responsible for its elimination [39].

These results were interpreted to indicate that agents affecting the amyloid-β peptide pathway may become possible neuroprotectants in glaucoma.

Guo et al. [40] examined three ways to target amyloid-β peptide in experimental glaucoma, including: (i) administration of a beta-secretase inhibitor to reduce amyloid-β peptide, (ii) administration of anti- amyloid-β peptide antibody to inhibit amyloid-β peptide deposition, and (iii) administration of Congo red to inhibit amyloid-β peptide aggregation and neurotoxic effects. These authors found that a combination of triple agents was most effective compared to other possible combination therapies.

However, the clinical outcomes of these amyloid-β peptide-based therapies have been disappointing so far [41]. It is possible that these agents may not target the misfolded Tau and neurofibrillary tangles (NFTs) that contribute significantly to the pathology of Alzheimer's disease [42].

■ Tau-dependent mechanisms in glaucoma

Tau protein is typically localized to axons where it binds to and stabilizes microtubules [43] in physiological conditions [43,44]. Aggregation of hyperphosphorylated tau induces impairments of axonal transport and neuronal degeneration [45]. NFTs are pathological hallmarks of Alzheimer's disease [46] and are comprised of intracellular filamentous hyperphosphorylated tau [47].

In the retina, tau is physiologically present and involved in axonal development and survival of RGCs [48]. Hyperphosphorylation of tau is increased in the retina of elderly persons [49]. In the vitreous from 8 glaucoma patients, tau levels were found to be quite high (113.6 ± 43.1 pg/ml) compared to levels in 13 control subjects (3.3 ± 3.2 pg/ml) despite low levels of beta-amyloid [33]. In surgically excised specimens from glaucoma patients, hyperphosphorylated tau, which is not seen in control specimens, is detected in the inner nuclear layer, most likely in horizontal cells [50]. In a rat glaucoma model with unilateral intraocular pressure (IOP) elevation, a marked tau increase is observed in the inner plexiform layer rather than GCL, suggesting that the primary site of tau accumulation is in RGC dendrites [51].

The use of transgenic mouse models harboring gene mutations (APP, PS1, or P301S) that cause familial early-onset Alzheimer's disease confirmed either the development of amyloid-β peptide plaques and/or neurofibrillary tangles with subsequent local neuroinflammation, characterized by microglial infiltration, astrogliosis, and disruption of inner parts of the retina [52,53].

In double transgenic APP/PS1 mice, hyperphosphorylation of retinal tau is accompanied by a preceding increase in calpain [54], and hypoxia induces abnormal calpain activation, which in turn increases ER stress-induced apoptosis in Alzheimer's disease pathogenesis [55]. Prominent activation of microglia was also observed in this transgenic model [56]. Microgliosis is induced even in the early phase of Alzheimer's disease, and is involved in the degradation of accumulated amyloid-β peptide. Additionally, microgliosis can induce neuronal inflammation, which may contribute to functional alterations in electroretinograms [57].

In the P301S mutant human tau transgenic mice, hyperphosphorylation and aggregation of tau were associated with reduced axonal transport in the optic nerve [58,59].

In Tg2576 transgenic mice, which carry a transgene derived from a Swedish family with early onset Alzheimer's disease, hyperphosphorylated tau is observed in the vicinity of amyloid-β peptide deposition [60]. In Tg4510 transgenic mice with the *P301L* mutation [61], activation of caspase induces tangle formation in neurons, and while tangle-bearing neurons are long-lived, soluble extracellular tau is remarkably toxic. Although soluble extracellular tau in the CSF can be detected in healthy individuals, Alzheimer's disease patients shows significant amounts of this protein [62,63]. Soluble extracellular tau is also toxic to cell-to-cell communication, disrupting synaptic plasticity and resulting in subsequent cognitive impairment [64-66]. Additionally, oligomeric extracellular tau can bind to APP [67], and APP knock-out mice were resistant to oligomeric extracellular tau-induced impairments in long term potentiation (LTP). Thus, these authors suggested that APP may be a therapeutic target against Alzheimer's disease. There is increasing interest in developing tau-based therapies for treating tauopathies including Alzheimer's disease [68].

Transcription factor EB (TFEB) is a molecule that plays a central role in cellular degradative processes. TFEB effectively reduces neurofibrillary tangle pathology and rescues behavioral and synaptic deficits and neuronal degeneration in the Tg4510 transgenic mouse [69]. Phosphatase and tensin homolog is a direct target of TFEB

and is required for TFEB-dependent aberrant Tau clearance. The specificity and efficacy of TFEB in mediating the clearance of toxic Tau species makes it an attractive therapeutic target for treating tauopathies. Consistent with this, selenomethionine (Se-Met), a major bioactive form of selenium (Se) with significant antioxidant capacity, has recently been reported to reduce the levels of total tau and hyperphosphorylated tau and to ameliorate cognitive deficits in younger triple transgenic Alzheimer's disease mice (three mutations associated with familial Alzheimer's disease genes; *APP Swedish*, *MAPT P301L*, and *PSEN1 M146V*) (3xTg-AD mouse) [70].

■ ApoE-dependent mechanisms in glaucoma

Apolipoprotein E (ApoE) is a very low-density lipoprotein, responsible in part for removing cholesterol from the bloodstream. In humans, the *APOE* gene exists as three different polymorphic alleles *(ε2, ε3 and ε4)*, which engender six different genotypes *(ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4)*. ε3 is the most common allele (77%) and *ε2* the least (8%) common allele [71]. *APOE* polymorphisms cause changes in cholesterol transport. The *ε4* allele frequency is about 15% in general populations, but is ca. 40% in Alzheimer's disease patients. Having one *ε4* allele increases the risk of developing Alzheimer's disease 3- to 4-fold compared to controls without *ε4* alleles [72,73]. In addition, receptors recognizing ApoE are also widely expressed in the Alzheimer's disease brain [74,75]. Although ApoE mediates amyloid-*β* peptide clearance by binding amyloid-*β* peptide and forming a stable complex, ApoE may also stimulate amyloid-*β* peptide aggregation and amyloid deposition, as well as tau hyperphosphorylation [76-79]. Moreover, among the mechanisms that might explain the effects of ApoE on the brain of Alzheimer's disease subjects, ApoE *ε4* and its receptors are also reported to be involved in APP trafficking and its processing to amyloid-*β* peptide [80]. Additionally, ApoE may mediate amyloid-*β* peptide cell internalization, by binding to the LDL receptor-related protein (LRP) [81].

A number of epidemiological studies also report that individuals with hypercholesterolemia have increased risk of developing Alzheimer's disease. The risks to develop Alzheimer's disease are significantly increased by having the *APOE ε4* genotype, which may influence cholesterol metabolism and the formation of cholesterol

oxidation products, known as oxysterols [82,83]. Inoue et al. [84] reported that glaucoma patients have elevated levels of ApoE in the aqueous humor using a multiplex bead immunoassay. Although several reports suggest that APOE genotype may not be a risk factor for glaucoma [85-87], the association of *APOE* allelic isoforms *(ε2, ε3, and ε4)* has been investigated as a possible risk for different forms of glaucoma [88]. POAG patients with lower IOP (38.0%) and higher IOP (34.2%) had higher expression of *ε4* alleles compared to controls (18.9%). The odds of *ε4* carriers having normal tension glaucoma were also significantly greater than for *ε3* homozygotes (odds ratio 2.45, 95% confidence interval [1.02-5.91]). Thus, inheritance of the *ε4* allele appears to increase risk for glaucoma.

Furthermore, two types of single-nucleotide polymorphisms (SNPs) of *APOE, APOE(-219G) and APOE(-491T)*, are associated with Alzheimer's disease, and modify the phenotype of POAG [89]. The presence of *APOE(-219G)* increased cupping of the optic disc, and exacerbated visual field impairments. *APOE(-491T)* interacts strongly with a SNP in the MYOC promoter, and is associated with IOP elevation or resistance to IOP-lowering therapy. Despite these positive reports, it is important to note that there are other results indicating that *APOE* genotypes do not constitute risk factors for developing glaucoma [85-87].

Although the association between ApoE and glaucoma pathogenesis remains uncertain, impairments in synthesis and transport of cholesterol in the retina have deleterious effects on retinal function, indicating the importance of cholesterol metabolism in the retina. In this context, involvement of 24(S)-hydroxycholesterol (24(S)-HC), the major cholesterol metabolite in brain, has also been investigated in the etiology of both Alzheimer's disease and glaucoma [90].

Table 1 summarizes the common characteristics between Alzheimer's disease and glaucoma.

Table 2 summarizes the common pathological features between glaucoma and Alzheimer's disease.

Neurosteroids and Alzheimer's disease

Neurosteroids are endogenous modulators generated in the nervous system. Neurosteroids are synthesized from cholesterol and are potent modulators of excitatory glutamatergic

Table 1: Common characteristics between glaucoma and Alzheimer's disease.

Methods	Outcome measure	Results and references
Epidemiology	Occurrence of glaucoma in AD patients Occurrence of AD in glaucoma patients	Increase [11-13] Increase [17-19] No increase [20,21]
Elecroretinogram	RGCs (especially M cells) in AD	Affected [14]
Optical coherence tomography	Nerve fiber layer thickness in AD	Significantly decreased [15,16]
Histology	Nerve fiber density Disappearance of RGC Reactive gliosis RGC density M-cells	Decrease in both AD and glaucoma [22] Disappearance of RGCs in both AD and glaucoma [22] Induction of reactive gliosis in the GCL in both AD and glaucoma [22] Decrease in both AD and glaucoma [23] Decrease in both AD and glaucoma [24]

Table 2: Common patholohical features between glaucoma and Alzheimer's disease.

Mechanism	Outcome measure	Results and references
1. Apoptosis	Apoptotic neuronal degeneration Caspase	Activated in glaucoma [29-31] Activated in glaucoma [29]
2. Amyloid-dependent mechanism	Amyloid β peptide (Abeta)	Increased in glaucoma [32] Caspase-3-mediated abnormal APP processing induced apoptosis in glaucoma [34,35]
	Presenilin 1 (PS1) and PS2	Gene mutataion induced abnormal amyloid precursor protein, and activated caspase-3-mediated abnormal APP processing in glaucoma model [34]
	Amyloid precursor protein (APP)	Neuroprotective in glaucoma model [33]
3. Tau-dependent mechanism	Tau	High level in vitreous of glaucoma patient [33] Accumulated in the RGC axon in the rat glaucoma model [51]
	Hyperphosphorylation of tau	Highly expressed in the aging human retina [49] Mainly localized in the horizontal cell in glaucoma patient retina [50]
4. Induction of Transgenic mouse	Characteristics of APP/presenilin 1 mouse	Age-dependent deposition of Abeta in the NFL [35] Age dependent increase in TUNEL-positive RGC [35] Microglial infiltration, astrogliosis of inner retina [52,53]
	Characteristics of P301S mutant human tau transgenic mice	Reduced axonal transport in the optic nerve [58,59]

and inhibitory GABAergic neurotransmitter systems [91,92]. As examples, 24(S)-HC and allopregnanolone (AlloP) are positive modulators of glutamate N-methyl-D-aspartate receptors (NMDARs) and GABA$_A$ receptors, respectively. Recently, neurosteroids have been considered as putative therapeutic agents for multiple neurodegenerative disorders [93-95].

■ Oxysterols

Oxysterols are divided into two classes: sterol-ring oxidized oxysterols such as 7-ketochoelsterol or 7α/β-hydroxycholesterol, and side-chain oxidized oxysterols such as 24(S)-HC, 25-hydroxycholesterol (25-HC), or 27-hydroxycholesterol (27-HC). In general, the former class is produced by reactive oxygen species (ROS), and the latter is enzymatically generated from cholesterol [96].

25-HC appears to be a weak partial agonist at NMDARs and has antagonizing effects against 24(S)-HC on NMDARs [97]. It has been reported that 25-HC induces apoptosis of neuronal cells at high concentrations [98]. Cholesterol 25-hydroxylase is the enzyme that catalyzes formation of this oxysterol. In Alzheimer's disease, 25-HC enhances amyloid-β peptide insertion into cell membranes (the peptide penetration into membrane by 25-HC was confirmed by experiments using artificial model membranes [99]), thus affecting mitochondria or endosomes, and subsequently leading to oxidative stress-mediated apoptosis [100]. Recently, 25-HC was reported to induce neuronal death in amyotrophic lateral sclerosis (ALS), another major neurodegenerative illness, via effects on neuronal apoptosis. Studies examining the potential role of 25-HC in ALS may be instructive for understanding the role of oxysterols in other neurodegenerative conditions such as Alzheimer's disease and glaucoma. Kim et al. [101] demonstrated that serum 25-HC concentrations were significantly higher in ALS patients (5.39 ± 1.94 ng/ml) than in controls

(4.27 ± 1.18 ng/ml). Serum 25-OH levels were negatively correlated with ALSFRSr score (the revised ALS functional rating scale score) (-0.591; 95% CI -5.385, -0.673; p = 0.014) by multivariate regression analysis. Additionally, 25-HC also triggered neuronal apoptosis by activation of the glycogen synthase kinase-3 beta (GSK-3β)/ liver X receptor (LXR) pathways in in vitro ALS models.

27-HC is a metabolite of cholesterol formed mainly in the periphery that is capable of passing into the brain. In contrast with 24S-HC, an effective inhibitor of the amyloid-β peptide formation [102], 27-HC increases amyloid-β peptide and oxidative stress in in vitro [103]. To date most information about 27-HC in neurodegenerative disorders comes from studies of ALS. A large and comprehensive genome-wide association study was performed in an attempt to find novel genetic variants and candidate genes for sporadic ALS, and reported that the gene coding for the enzyme CYP27A1 (cholesterol 27-hydroxylase) that converts cholesterol into 27-HC, may be a risk factor for sporadic ALS [104]. Abdel-Khalik et al. [105] quantified non-esterified cholesterol and its metabolites in CSF and serum from ALS patients compared with a group of healthy controls using LC/MS. Comparing results of cholesterol metabolites between CSF and serum, it was suggested that impaired activity of CYP27A1 may lead to a failure of the CNS to remove excess cholesterol, which may in turn be toxic to neuronal cells, compounded by a reduction in neuroprotective 3β,7α- dihydroxycholest-5-enoic acid and other LXR ligands. Mateos et al. [106] demonstrated that 27-HC decreases activity-regulated cytoskeleton-associated protein (Arc) levels as well as NMDAR expression in rat primary hippocampal neurons. Arc is thought to contribute to the molecular mechanisms underlying learning and memory. Findings with 27-HC noted above promoted interest in measuring plasma levels of 27-HC in ALS patients. Wuolikainen et al. [107] measured the level of 27-HC using isotope dilution mass spectrometry and found that 27-HC was significantly lower in male ALS patients (p=0.008) compared to male controls (fold ALS/controls: 0.81). The lower levels could not be explained by a correlation with cholesterol. When testing 27-HC in males normalized against BMI, the difference was still significant (p=.03).

Cholesterol is mainly removed from the brain by enzymatic conversion into 24(S)-HC, which diffuses across the blood–brain barrier. Therefore, 24(S)-HC is proposed to be a marker of brain cholesterol metabolism. 24(S)-HC is synthesized from cholesterol by CYP46A1 (cholesterol 24-hydroxylase), a brain and neuron specific enzyme, coded by *CYP46A1* gene. Although 24(S)-HC involvement in the etiology of Alzheimer's disease has also been investigated [108], the association of 24(S)-HC with Alzheimer's disease remains equivocal. There are a number of reports showing that 24(S)-HC levels are elevated or lowered in CSF and plasma of Alzheimer's disease patients as compared to control [109-111], although some of these discrepancies may relate to stage of illness. Papassotiropoulos et al. [109] reported that 24(S)-HC in CSF is significantly elevated at early stages of Alzheimer's disease compared to control (Alzheimer's disease vs. control = 2.6 ± 1.1 ng/ml vs. 1.6 ± 0.6 ng/ml, p<0.001) using combined gas-chromatography and mass spectrometer. It is possible that elevated cholesterol levels induced by myelin destruction causes an increase in 24(S)-HC level. By contrast, patients with advanced Alzheimer's disease had significantly reduced 24(S)-HC levels in the plasma [112]. As Alzheimer's disease progresses, 24S-HC levels in plasma and CSF decline, possibly reflecting extensive neuronal loss.

CYP46A1 may be preferentially expressed in degenerating neurites surrounding senile plaque in brains of Alzheimer's disease patients [102]. Furthermore, association of CYP46A1 polymorphisms with risk of Alzheimer's disease and elevated amyloid-β peptide load has been reported [113,114]. These results suggest that 24(S)-HC abnormally synthesized via CYP46A1 may be more directly involved in Alzheimer's disease pathogenesis.

Conversely, upregulation of CYP46A1 is also found to be neuroprotective in animal models of Huntington's disease, another major neurodegenerative illness [115]. Under physiological conditions, 24(S)-HC derived from neurons may signal astrocytes to increase production of lipidated ApoE particles in order to supply neurons with cholesterol during synaptogenesis or neuritic remodeling [116]. Moreover, alterations in the transcriptional regulation role of 24(S)-HC on ApoE-mediated cholesterol efflux may affect the progression of neurodegenerative diseases including Alzheimer's disease [116]. Although there is still controversy, CYP46A1 and 24(S)-HC have possibilities to exert neuroprotection in neurodegenerative diseases.

■ Allopregnanolone

AlloP is an endogenous neurosteroid synthesized in the CNS from cholesterol, and a potent and effective positive modulator of the major inhibitory neurotransmitter, GABA. Previous studies reported that astrocytes or other glial cells synthesized AlloP under pathological conditions [117-119]. Recently, increasing evidence indicates that principal excitatory neurons synthesize GABAergic neurosteroids [120]. In the process of endogenous AlloP synthesis, cholesterol translocation to mitochondrial inner membrane by translocator protein 18 kD (TSPO) [121] and the catalytic reaction by 5α-reductase (5aRD) [122,123] are considered rate-limiting steps. AlloP potentiates the activity of GABA$_A$ receptors [124], and exerts neuroprotective properties both in *in vitro* cell culture [125-128] and *in vivo* animal models [129-131]. AlloP also increases myelination [132-134], enhances neurogenesis [135], decreases inflammation [134,136,137], and reduces apoptosis [138-140]. Deficits in AlloP could induce excitotoxicity [141-144], neurodegeneration [145-147], dysregulation in myelination [148,149], neurogenesis [150,151], apoptosis [152,153], and inflammation [154], possibly contributing to Alzheimer's disease pathophysiology. A reduction of AlloP in temporal cortex of Alzheimer's disease patients is reported to be negatively associated with disease progression [155].

The presence of the *APOE ε4* allele, a risk factor for developing Alzheimer's disease, is associated with reduction in AlloP levels. AlloP median levels in temporal cortex are significantly decreased in patients homozygous or heterozygous for the *APOE ε4* allele (2.86 ng/g, n=36) compared to patients not carrying an *APOE ε4* allele (5.23 ng/g, n=44) (Mann Whitney p=0.04) [156].

Wang et al. [135] showed that AlloP significantly increased proliferation of hippocampus-derived neural progenitor cells and cerebral cortex-derived neural stem cells. Additionally, AlloP upregulated genes promoting mitosis and downregulated genes that repress cell proliferation. Proliferation by AlloP was inhibited by nifedipine, an antagonist of L-type voltage-gated calcium channels, suggesting that AlloP induces proliferation of neural progenitor cells and neural stem cells via a mechanism dependent upon L-type calcium channels. The 3xTgAD mouse carries mutations in two human familial Alzheimer's disease genes (*APP$_{Swe}$*, *PS1$_{M146V}$*) and one frontal temporal dementia-linked tau mutation (*tauP301L*), and manifests age-dependent neuropathology that includes both β-amyloid plaques and neurofibrillary tangles. Wang et al. [95] examined the neuroproliferative effects of AlloP in the hippocampal subgranular zone (SGZ) and the reversibility of learning and memory deficits in 3xTgAD transgenic mice. At 3 months of age, AlloP induced a significant increase in progenitor cell proliferation with subcutaneous injection of 10 mg/kg AlloP in 3xTgAD male mice (P < 0.05). AlloP also ameliorated cognitive impairment with learning and memory defects improving to normal levels. These findings suggest that AlloP may be able to prevent or delay cognitive deficits associated with early onset Alzheimer's disease [155].

The same research group [157] also examined the efficacy of potential treatment regimens with AlloP in the 3xTgAD male mouse model. Three different AlloP treatment regimens were compared; (1) 1/month single injection, (2) 3/week x 3 months and (3) 1/week x 6 months paradigms. In each regimen, AlloP was subcutaneously injected at 10 mg/kg body weight. Results indicate that AlloP administered 1/week for 6 months was most effective in increasing survival of newly generated neurons and simultaneously reducing amyloid-β peptide pathology in 3xTgAD male mice.

Taken together, these findings provide preclinical evidence that AlloP administration has the potential to promote regeneration and decrease amyloid-β peptide production [157].

Glaucoma and Neurosteroids

■ Oxysterols

We did not find any papers examining an association between 25-HC and glaucoma. The gene expression profile analysis of human trabecular meshwork cells revealed that the 27-HC synthesizing enzyme (CYP27A1) was differentially expressed in response to steroid [158]. However, functional studies of CYP27A1 in glaucoma remain to be done.

Fourgeux et al. [159] found that the frequency of *rs754203* SNP in the *CYP46A1 intron 2* was significantly higher in POAG patients compared to controls (POAG vs. control = 61.3% vs. 48.3%, p<0.05). However, a subsequent epidemiologic study failed to replicate the association between *rs754203* SNP and POAG [160], making it unclear whether there is a genetic association between *CYP46A1* and glaucoma.

CYP46A1 specifically located in RGCs, the cells most affected by elevated IOP in glaucoma. This localization of CYP46A1 supports the idea that 24(S)-HC may play a role in glaucoma pathogenesis. Fourgeux et al. [161] reported that CYP46A1 expression was induced early in response to IOP elevation, but CYP46A1 up-regulation is only transient and returned to baseline within a few days in a rodent experimental glaucoma model.

In our previous study, we examined the role of 24(S)-HC in glaucoma using a rat *ex vivo* model in which pressure was adjusted 10 mmHg and 75 mmHg for 24 hours to simulate physiological IOP and conditions during an acute angle closure glaucoma attack, respectively. This glaucoma model has the advantage that it avoids baseline ischemic degeneration and the influence of circulating steroids [162,163]. In this *ex vivo* model, we found that 24(S)-HC production is increased in RGCs, while cholesterol concentration is reduced following pressure elevation [164].

Based on studies in hippocampus, Sodero and colleagues [165,166] proposed a model, in which stress- and aging-induced activation of glutamate receptors promotes translocation of CYP46A1 from the endoplasmic reticulum to the cell surface, resulting in acceleration of 24(S)-HC synthesis, and subsequent induction of neuronal survival pathways. Consistent with this, we found that administration of 1 μM 24(S)-HC diminished apoptotic RGC death and axonal injury in the hyperbaric condition in our *ex vivo* model [164]. We also observed that the CYP46A1 inhibitor, voriconazole, was toxic at 10 mmHg and 75 mmHg [164]. These findings indicate that 24(S)-HC is likely an endogeneous neuroprotectant under glaucomatous conditions.

However, the neuroprotective effects of 24(S)-HC are contradictory, because it has been reported that this oxysterol is a potent allosteric NMDAR modulator in CNS, and would thus be expected to promote excitotoxic damage [167,168]. Previous studies found that 24(S)-HC plays a complex role under pathological conditions [169] with another endogenous oxysterol, 25-HC, dampening the effects of 24(S)-HC on NMDAR [97,170]. In addition, cholestane-3β, 5α, 6β-triol, another oxysterolk is a negative allosteric modulator of NMDARs [170,171]. Thus, further studies are needed to understand the possible interactions among oxysterols under pathological conditions such as pressure elevation.

■ Allopregnanolone

In our acute model [172,173], pressure elevation induced expression of two key participants in AlloP synthesis, TSPO and 5aRD (mostly type II), in RGC. Furthermore, several other acute neuronal stressors such as acute forced swim [174] ammonia [175], ethanol [176] and acetaldehyde [177], induce AlloP synthesis in the brain. Since IOP elevation is a form of acute stress, the findings with elevated pressure in the retina are consistent with what has been observed elsewhere in brain.

Atriol, an agent that preferentially inhibits TSPO [178], and dutasteride, a broader spectrum 5aRD inhibitor, suppressed pressure-induced AlloP synthesis and induced excitotoxic retinal degeneration in hyperbaric condition. These findings indicate that upregulation of endogenous AlloP and GABA$_A$ receptor activation are critical to maintain the integrity of the retina during the stress of pressure elevation. Exogenous administration of AlloP also inhibited axonal swelling, indicating that AlloP can be a neuroprotectant against glaucomatous changes. Picrotoxin, a GABA$_A$ receptor antagonist, attenuated the neuroprotective effects of AlloP, supporting the hypothesis that neuroprotection by AlloP involves GABA$_A$ receptors.

The TSPO agonist, Ro5-4864, has been found to be neuroprotectant in a mouse model of Alzheimer's disease (3xTgAD mice) [179]. Furthermore, the TSPO ligand, PK11195, has at least partial agonist activity at TSPO, depending upon cell type and drug concentration [180,181], and, in the rat *ex vivo* glaucoma model, PK11195 clearly enhanced actions mediated by TSPO, preventing pressure-induced RGC injury and apoptotic RGC death. Thus, PK11195 or other TSPO agonists may be useful agents in protecting RGCs from pressure-induced excitotoxic degeneration via effects on AlloP synthesis [182].

Table 3 summarizes the neurosteroids in Alzheimer's disease and glaucoma. **Figure 1** depicts key steps and enzymes involved in the synthesis of AlloP and 24(S)-HC from cholesterol.

Conclusion

Elevated IOP is the predominant risk factor for glaucoma, and, to date, IOP lowering therapies

Table 3: Neurosteroids and oxysterols in Alzheimer's disease and glaucoma.

Neurosteroids and their generating enzymes	Alzheimer disease (results and references)	Glaucoma (results and references)
25-Hydroxycholesterol (25-HC)	Upregulate Abeta insertion into membrane [99]	-
	Promoted peptide penetration into membranes [99]	-
	Induce oxidative stress and apoptosis [98,100]	-
27-Hydroxycholesterol (27-HC)	Dampen beneficial effects of 24(S)-HC inhibiting Abeta formation [102]	-
	Increases Abeta and oxidative stress [103]	-
	Decrease activity-regulated cytoskeleton-associated protein (Arc) levels [106]	-
27-HC generating enzyme (CYP27A1)	Risk factor for sporadic ALS [104]	-
24(S)-Hydroxycholesterol (24(S)-HC)	Efficient inhibitor of the formation of Abeta peptides [102]	Increase in pressure-dependent manner [164]
	Increase in CSF of early stages [109] and decrease in plasma and CSF of advanced stage [112]	Neuroprotective agaist IOP elevation [164]
24(S)-HC generating enzyme (CYP46A1)	Preferentially expressed in degenerating neurites surrounding senile plaque of AD [102]	Increase in pressure-dependent manner [164]
	CYP46A1 polymorphisms increase risk of AD and elevated Abeta load [113,114]	
	CYP46A1 activation results in cholesterol loss and subsequent stimulation of neuroprotection in excitotoxicity in hippocampus [165,166].	
Allopregnanolone (AlloP)	Decrease in temporal cortex in AD patient [155]	Increase in pressure-dependent manner [172,173]
	Decrease with the presence of the APOE4 allele, a risk factor for late onset AD) [156]	Neuroprotective agaist IOP elevation [172,173]
	Increase proliferation of neural progenitor cells in transgenic mouse (APP/PS1/P301L) [95,135,155]	
AlloP generating sequence (TSPO,α-Reductase)5	TSPO agonist Ro5-4864 is neuroprotective in a mouse model of Alzheimer's disease (3xTgAD) [179]	Increase in pressure-dependent manner [173]
		PK11195, TSPO agonist, is neuroprotective against pressure-dependent injury [173]

are the only proven method to treat glaucoma. However, recent evidence indicates that the progression of RGC death cannot be prevented despite effective lowering of IOP. Therefore, alternative treatments will likely be required to delay or prevent progressive RGC damage. The term neuroprotection implies mechanisms that protect neurons from apoptotic or necrotic degeneration, and neuroprotection in glaucoma is aimed at protecting RGC that are damaged by glaucomatous processes. Based on common pathogenetic features between Alzheimer's disease and glaucoma, several possibilities exist to develop novel therapeutic strategies. Neurosteroids and oxysterols are synthesized from cholesterol in the CNS and offer a potential new avenue for treatment development. Among the neurosteroids,
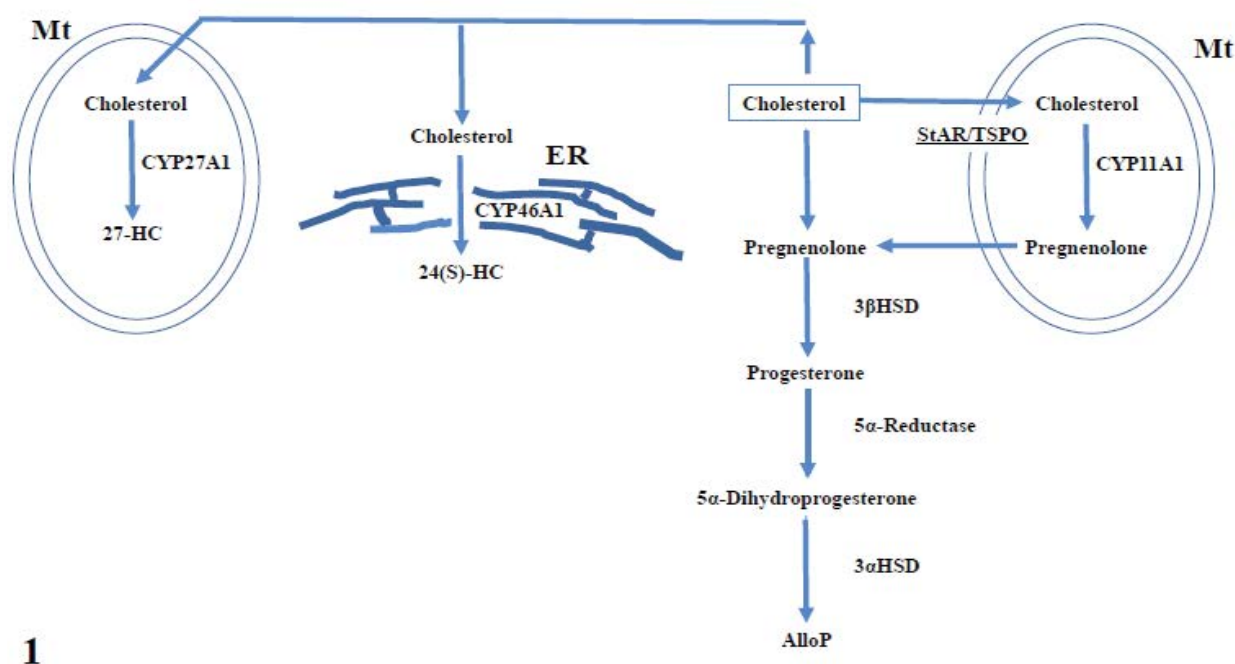
Figure 1: Depicts key steps and enzymes involved in the synthesis of AlloP, 24(S)-HC and 27-HC from cholesterol. CYP46A1 and CYP27A1 catalyze the synthesis of 24(S)-HC and 27-HC, respectively. CYP46A1 and CYP27A1 are localized in endoplasmic reticulum (ER) and mitochondria (Mt), respectively. AlloP is generated from cholesterol by a sequential action of 3β-hydroxysteroid dehydrogenase (3βHSD), 5α-reductase (5aRD) and 3α-hydroxysteroid dehydrogenase (3αHSD). Factors that differentially regulate the synthesis of 24(S)-HC, 27-HC and AlloP from cholesterol in retina and brain are not well understood [182].

24(S)-HC and AlloP are positive modulators of NMDA and $GABA_A$ receptors, respectively. In Alzheimer's disease and aging models, upregulation of CYP46A1, the 24(S)-HC generating enzyme, appears to be neuroprotective. Additionally, AlloP enhances neurogenesis, decreases inflammation, and reduces apoptosis, and thus could prevent neurodegenerative and cognitive deficits associated with Alzheimer's disease. In our *ex vivo* glaucoma model, pressure loading increases activation of CYP46A1 and 24(S)-HC synthesis, resulting in protection of RGC against pressure-induced damage. AlloP production is also increased in hyperbaric conditions, and also exerts neuroprotection against IOP elevation-induced axonal injury through mechanisms that are likely distinct from 24(S)-HC. As the next step of future research, the effects of these neurosteroids on cognitive function in transgenic mice modeling Alzheimer's disease and tau aggregation should be examined with paralleled studies in their retinas in order to determine the possible link between Alzheimer's disease and glaucoma. Further work is also needed to determine whether AlloP and 24S-HC are neuroprotective in different *in vivo* Alzheimer's disease models. Following from commonalities in the pathophysiology of glaucoma and Alzheimer's disease, it will also be interesting to determine how the retinas of mice expressing genes associated with Alzheimer's disease respond to changes in IOP and the effects of neurosteroids and oxysterols. Taken together, available data suggest that neurosteroids and oxysterols warrant further consideration as potential therapeutic approaches in both glaucoma and Alzheimer's disease.

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