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Review

The Pathological Effects of Circulating Hydrophobic Bile Acids in Alzheimer's Disease

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Abstract. Recent clinical studies have revealed that the serum levels of toxic hydrophobic bile acids (deoxy cholic acid, lithocholic acid [LCA], and glycoursodeoxycholic acid) are significantly higher in patients with Alzheimer's disease (AD) and amnestic mild cognitive impairment (aMCI) when compared to control subjects. The elevated serum bile acids may be the result of hepatic peroxisomal dysfunction. Circulating hydrophobic bile acids are able to disrupt the blood-brain barrier and promote the formation of amyloid-β plaques through enhancing the oxidation of docosahexaenoic acid. Hydrophobic bile acid may find their ways into the neurons via the apical sodium-dependent bile acid transporter. It has been shown that hydrophobic bile acids impose their pathological effects by activating farnesoid X receptor and suppressing bile acid synthesis in the brain, blocking NMDA receptors, lowering brain oxysterol levels, and interfering with 17β-estradiol actions such as LCA by binding to E2 receptors (molecular modelling data exclusive to this paper). Hydrophobic bile acids may interfere with the sonic hedgehog signaling through alteration of cell membrane rafts and reducing brain 24(S)-hydroxycholesterol. This article will 1) analyze the pathological roles of circulating hydrophobic bile acids in the brain, 2) propose therapeutic approaches, and 3) conclude that consideration be given to reducing/monitoring toxic bile acid levels in patients with AD or aMCI, prior/in combination with other treatments.

Keywords: Alzheimer's disease, blocking NMDA receptors, blood-brain barrier, declining cognitive function, liver dysfunction, serum bile acids

INTRODUCTION

As Alzheimer's disease (AD) is becoming a major public health threat, there is an urgent unmet need to develop new therapies for AD [1]. As a result, several agents have been tested in clinical trials over the years with disease modifying therapies (DMTs) comprising more than 60% of the agents, and symptomatic cognitive enhancing agents forming about 30% of drugs [1–4]. DMTs refer to agents that either prevent, delay, or slow the progression of the disease [5]. DMTs themselves can be categorized into two groups: immunotherapy (mostly using antibodies) or small molecules [1]. There were 17 DMT agents in phase III of clinical trials in 2018, and eight agents were terminated in 2019 [4]. Preventing tau aggregation has been another approach in DMT. Leuco-methylthioninium bis(hydromethanesulfonate) (LMTM), a stable

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reduced form of the methylthioninium moiety, acts as a selective inhibitor of tau protein aggregation. LMTM did not show efficacy in a phase III clinical trial [6]. As another example, intranasal insulin showed significant improvements in learning, memory, and cognitive function in a pilot study of mild to moderate AD [7]. Then a clinical trial (NCT01767909) examined the effects of intranasally-administered insulin on cognition, entorhinal cortex, and hippocampal atrophy, and cerebrospinal fluid (CSF) biomarkers in amnestic mild cognitive impairment (aMCI) or mild AD. This trial completed, but no cognitive or functional benefits were observed with intranasal insulin treatment over a 12-month period [8]. Device malfunction was reported at the early stages of the trial [9].

With respect to symptomatic cognitive enhancing agents (such as donepezil, a cholinesterase inhibitor), these generally do not tackle the disease's underlying mechanism. One of the drugs in this class, ITI-007, entered phase III clinical trial with the aim of improving neuropsychiatric symptoms (agitation) in AD patients. However, the trial was terminated in 2018, as interim data analysis revealed that the trial would not meet the primary outcome. On the other hand, suvorexant was tested in patients with AD for the treatment of insomnia [10]; and the drug was approved by the FDA for the indicated use.

The above observations show that there are other important disease underlying mechanisms such as oxidative stress [11] that should be considered in developing therapeutic agents for AD. This paper aims to expand on this point and present that altered bile acid metabolism could play major roles in developing AD.

LIVER DISFUNCTION CONTRIBUTING TO AD

A previous study found that the expression of peroxisomal D-bifunctional protein (D-BP) decreased significantly in the liver of patients with AD compared to control subjects [12]. This enzyme catalyzes conversion of tetracosahexaenoic acid into docosahexaenoic acid (DHA). Therefore, defective D-BP activity impairs DHA biosynthesis in the liver of patients with AD. It should be noted that DHA is found mainly as phospholipid species such as 38 : 6 and 40 : 6 in phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylserine (PS) [13], and lysophosphatidylcholine (LPC-DHA: LPC 22 : 6, CHEBI:73873, Mw: 567.7) [14]. Interestingly, association of blood lipids and AD has been shown [15, 16]. Therefore, reduced synthesis of DHA in the liver (due to defective D-BP activity) would lead to reduced serum lipids, such as PC 38 : 6 (CHEBI:74963, CAS: 59403-54-2), and PC ae C40 : 6 (CHEBI:86252) as shown in patients with AD [15]. Importantly, *in vivo* studies found that DHA in the form of LPC-DHA is transferred across the blood-brain barrier (BBB) by a member of major facilitator superfamily transporters (Mfsda2) [17]. Furthermore, dietary LPC-DHA significantly increased brain DHA content and improved brain function in adult mammals, but not DAH as free acid [18]. Therefore, reduction of DHA synthesis in the liver of patients with AD would reduce plasma levels of LPC-DHA (normally 21.5 μM) [14]. Reduced plasma levels of LPC 18 : 2 have been reported in patients with AD [15] as well as LPC-DHA in these patients [19]. Now the important point is that D-BP is also involved in the biosynthesis of bile acids (shown schematically in Fig. 1) [20].

Figure 1A shows bile acid biosynthesis by classic and acidic pathways in the liver. In the classic pathway, cholesterol is transported into endoplasmic reticulum (ER) and oxidized to 7α-hydroxyl-4-cholesten-3-one (C4) by 3βHSD7. At this point, part of C4 is hydroxylated at position 12 by CYP8B1 to yield 7α, 12α, dihydroxyl-4-choleste-3-one. Both C4 and 7α, 12α, dihydroxyl-4-choleste-3-one are modified by several enzymes (AKR1D1, 3αHSD, CYP27A1, and BACS) to form 3α, 7α, dihydroxy-5β-cholestanoyl-CoA and 3α, 7α, 12α, trihydrohydroxy-cholestanoyl-CoA, respectively, which then are transported into peroxisome by the 70-kDa peroxisome membrane protein (PMP70). Figure 1B presents the side cleavage of 3α, 7α, 12α, trihydrohydroxy-cholestanoyl-CoA ((*25R*)-THC-CoA). It can be seen that D-BP converts (*24E*) THC:1-CoA to 24-keto-(*25R*)-THC-CoA at two steps. Figure 1B also depicts an alternative pathway for side chain oxidation employing L-bifunctional protein (L-BP) and AMACR enzymes. [21]. Therefore, when D-BP is not functioning, L-BP can step in and continue the formation of bile acids, but it appears that the activity of L-BP is much less than D-BP in bile acid biosynthesis. As in D-BP deficient patients, the hepatic ratio of C_{27}/C_{24} bile acid increased to 1.45 compared to 0.04 in control subjects [20]. If L-BP was as active as D-BP, the C_{27}/C_{24} bile acid would not have significantly increased in D-BP deficient patients. The side chain cleavage of

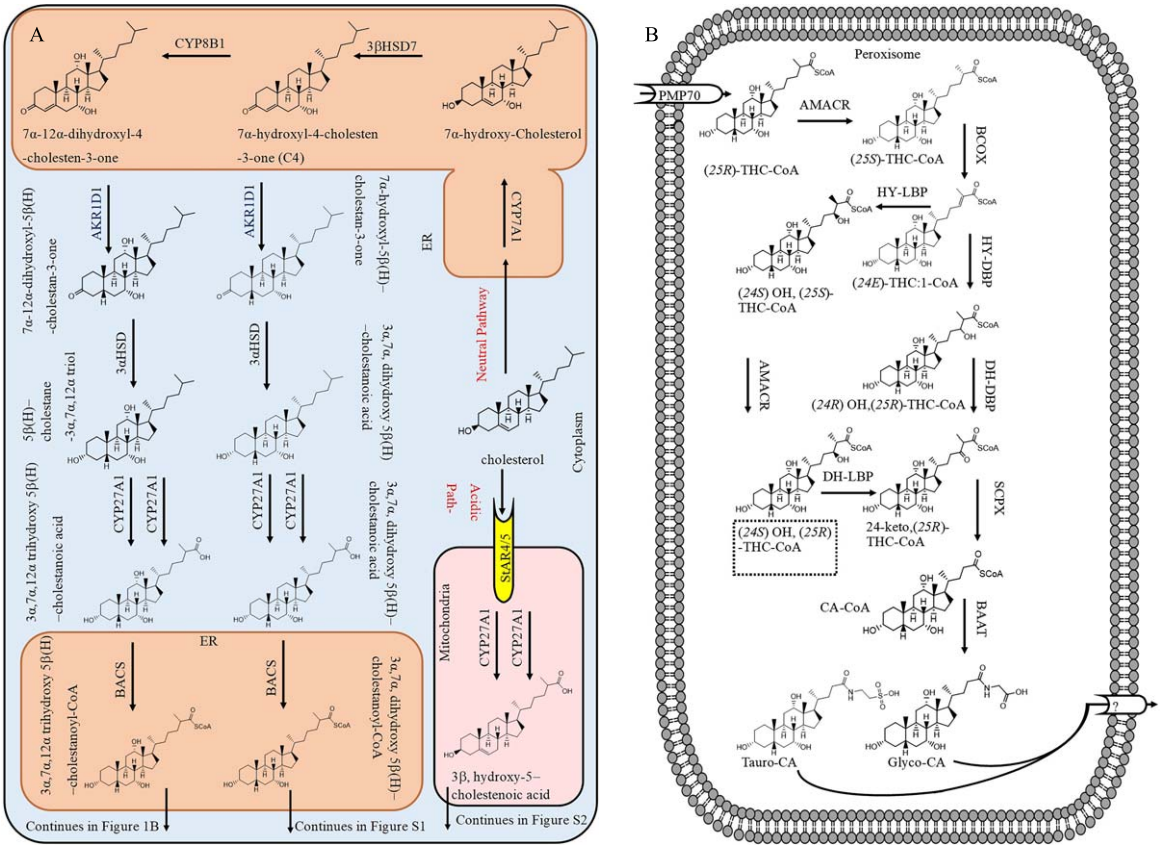

Fig. 1. The biosynthesis of bile acids from cholesterol by the neutral or acidic pathway A) in the cytoplasm and B) completion in peroxisomes (only for biosynthesis of conjugated cholic acid).

3α, dihydrohydroxy-cholestanoyl-CoA is presented in Supplementary Figure 1, which also shows the formation of conjugated CA and CDCA.

The acidic pathway (also known as alternative pathway) contributes about 10% of total bile acid production [22]. In addition, normally cholesterol is converted to other forms such as 27 hydroxy-cholesterol (3α, hydroxyl-5-cholestenoic acid) in extra-hepatic tissues or 24(S) hydroxycholesterol in the brain and it is transported into the liver by blood circulation for conversion mainly to CDCA via the acidic pathway [23]. This pathway can become the major bile acid production route when the classic pathway in the liver is not functioning normally due to deficiencies of enzymes such CYP7A1 [24, 25]. In one of the acidic pathways, which occurs in mitochondria, cholesterol is oxidized to 3β, hydroxy-5-cholestenoic acid by CYP27A1 at two steps and released to cytosol (Fig. 1A). Then, 3β, hydroxy-5-cholestenoic acid is uptaken into the ER and converted to 3β, 7α, dihydroxy-5-cholestenoic acid by CYP7B1. Afterwards, in one of metabolic pathways in the liver, 3β, 7α, dihydroxy-5-cholestenoic acid is modified to 3β, 7α, dihydroxy-5β-cholestanoyl-CoA (Supplementary Figure 2), which then is transported into peroxisome by PMP70 for side chain cleavage (Supplementary Figure 1).

Defective D-BP will affect levels of bile acids in the blood too [26], as these have been also observed in AD patients (increased plasma levels of GCA, GDCA, GCDCA) [27]. It is believed that defective D-BP results in the accumulation of C27 intermediate bile acids such as 3α,7α,12α trihydroxycholestanoic acid (THCA), 3α,7α dihydroxycholestanoic acid (DHCA), and OH-THCA in the liver, leading to cholestasis and consequent hepatic injury [20]. This hepatic injury may also result in further changes in the serum/plasma lipid levels. For example, six lipid metabolites were found to be cholesteryl esters (ChE 32 : 0, ChE 34 : 0, ChE 34 : 6, ChE; 33 : 6, ChE 40 : 4, ChE 32 : 4) that are reduced in plasma of patients with

AD [28]. In another study, a combination of 24 blood metabolites classified AD patients with > 70% accuracy [29]. This study identified a blood lipid signature that predicts AD progression.

The neurological functions of bile acids have been reviewed recently [30–32], and neurological impairment has been reported with increased blood bile acid levels [33]. Air pollution [34] and genetic variations [35] may alter bile acid metabolism in the body and promote developing AD. In addition, elevated serum/plasma levels of toxic bile acids such as lithocholic acid (LCA) may originate from defective D-BP in the liver of patients with AD, which could play major roles in developing AD. This is further explained in the following sections.

HIGH BILE ACID SERUM/PLASMA LEVELS IN PATIENTS WITH AD

Clinical studies have indicated increased serum bile acid levels in patients with AD compared to control healthy subjects [15, 27, 36], as well [15] as increased urinary conjugated primary bile acid (taurochenodeoxycholic acid (TCDCA)) and conjugated secondary bile acid (taurodeoxycholic acid (TDCA)) levels [37]. It was originally reported by Greenberg et al. (2009) that the plasma levels of glycocholic acid (GCA), glycodeoxycholic acid (GDCA), and glycochenodeoxycholic acid (GCDCA) increased in patients with AD compared to control subjects [27]. Few years later, Mapstone et al. (2014) found that the serum levels of glycoursodeoxycholic acid were higher in patients with aMCI/AD compared to control subjects [15]. This was followed by the work of Olazaran et al. in 2015 that screened 495 plasma metabolites in patients with AD and normal control subjects. They found that plasma levels of deoxycholic acid (DCA) and LCA significantly increased in patients with AD compared to control subjects [38]. These observations were confirmed by Marksteiner et al. (2018), who reported that the plasma levels of LCA increased to 50 ± 6 nM, which was significantly higher compared to control subjects (32 ± 3 nM) [39] It should be noted that LCA is nontoxic to neurons at concentrations below 25 μM [40]. Interestingly, the levels of LCA were higher in the brain of patients with AD compared to control subjects, suggesting that LCA may accumulate in the brain perhaps due to increased LCA serum levels, leading to declining of cognitive function in patients with AD [41]. MahmoudianDehkordi et al. (2019) found that the serum levels of secondary bile acids (DCA and its glycine and taurine conjugates) were significantly higher in patients with AD than control subjects, although serum levels of primary bile acids were significantly lower than control subjects [42]. While Shao et al. (2020) discovered that plasma levels of cholic acid were significantly higher than control subjects [36]. Organic anion transporter polypeptide 1 (OATP1) expressed in the choroid plexus and organic anion transporter polypeptide 2 (OATP2) expressed at the BBB both mediate the transport of bile acids from the systemic circulation into the brain [31]. Significant positive correlations have been observed between serum and brain bile acid concentrations for CA, CDCA, DCA, and taurocholic acid (TCA). LCA levels were below the limit of quantification in the brain [43].

On the other hand, Pan et al. (2017) did not find a significant difference in plasma levels of LCA between control subjects and AD patients, and in fact the levels of LCA in AD patients were lower (44 ± 10 nM) than in control subjects (63 ± 19 nM). Also, there was no significant difference in brain levels of LCA between AD patients (0.05 ± 0.01 nmol/g) and control subjects (0.06 ± 0.02 nmol/g) [44]. It should be noted that serum/plasma levels of LCA in young healthy subjects is 19.9 ± 1.1 nM [45]. Therefore, these observations suggest that serum levels of LCA increase with age, which may have a role in gradual declining of cognitive function in non-cognitively impaired healthy elderly subjects [46]. Furthermore, these observations suggest that increased serum/plasma bile acid levels may happen at some stage of the disease and then may decrease due to other metabolic dysfunctions in the patients.

Total serum bile acids for general population is 3.8 ± 0.2 μM [47]. However, total serum bile acid levels less than 1.5 μM are considered normal [48], but total serum bile acid level of 14 μM is considered the normal upper limit [48]. The total serum bile acid levels can be as high as 100 times of normal levels [48]. Values of total serum bile acid ranged from 0.3 to 9.8 μM in 216 pregnant women [49], while total serum bile acid levels increased from 4.2 μM in control subjects to 6.5 μM in patients with AD [39]. Therefore, total serum bile acid levels do not fall beyond normal upper limit in patients with AD, suggesting serious pathological effects are not expected, but chronic and steady declining effects on organs.

Patient and healthy subject data indicate that during aging the serum levels of LCA increase, however, the increase in AD patients is more noticeable [39, 47].

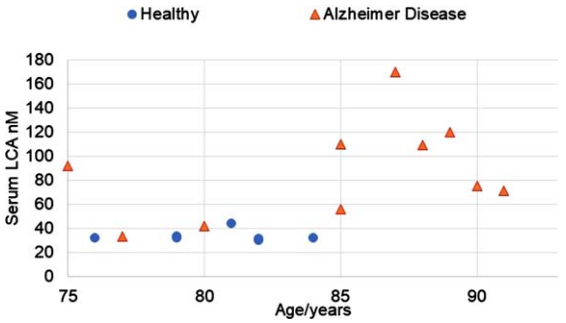

Fig. 2. Serum lithocholic acid (LCA) levels versus the age for healthy subjects ( ) and AD patients ( ), demonstrating that LCA serum levels matches healthy subject before age 85. Raw data was obtained from Marksteiner et al. (2018) [39].

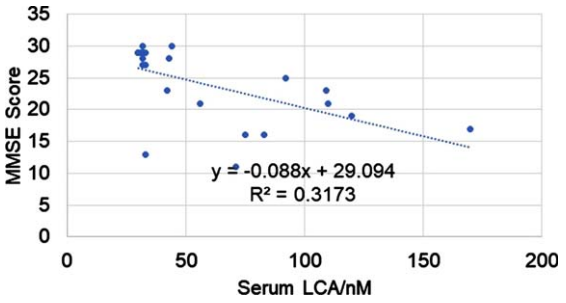

Fig. 3. Serum lithocholic acid (LCA) levels versus MMSE score ($n = 23$), population includes healthy subjects, subjects with mild cognitive impairment and patients with AD, suggesting that generally MMSE levels tend to decrease by increasing serum LCA levels. Raw data was obtained from Marksteiner et al. (2018) [39]. Further studies are required to demonstrate this in larger populations.

For example, serum LCA levels were 7.50 ± 0.38 nM in healthy subjects aged between 19 and 65 years [47], while this increased to 32 ± 3 nM in healthy subjects aged 77 ± 1.2 (SEM), and 50 ± 6 nM in AD patients aged 79 ± 2.0 years [39]. Based on the available data in a clinical study, we plotted the serum LCA versus age for healthy subjects and AD patients (Fig. 2, raw data was obtained from Marksteiner et al. [39]). With age serum LCA level increases for AD patients. Also, Fig. 3 (raw data was obtained from Marksteiner et al. [39]) depicts Mini-Mental State Examination (MMSE) versus serum LCA showing a decrease in MMSE when serum LCA concentration increases.

HYDROPHOBIC BILE ACIDS DISRUPT THE BLOOD-BRAIN BARRIER

The BBB is a selective barrier that eclipses the brain and isolates it from the circulating blood. It is composed of the capillary basement membrane (BM), astrocyte end feet ensheathing the vessels, and pericytes embedded within the BM. It represents a major barrier for drug permeation especially those which are highly hydrophilic and have molecular mass greater than 400 Da. Hydrophobic bile acids such as DCA alter the permeability of the BBB to molecules that otherwise normally do not cross the BBB [50, 51]. Therefore, DCA potentially expose the brain neurons to harmful metabolites/chemicals in the blood (Fig. 4). Interestingly, DCA caused significant reduction in the brain mass of rats, [50] a phenomenon that is observed in patients with AD compared to cognitively normal subjects [52]. Peripapillary edema and alteration in tight junctions could be the reasons for increased BBB permeability by DCA [53]. In addition, bile acids are capable of damaging the BBB by their detergent and lytic effects on the cell membrane [53]. Cerebral endothelial cells form tight junctions with adjacent cells via interactions of the tight junction proteins: occluding, claudin-5, and intracellular docking proteins ZO-1and ZO-2. Bile acids (DCA and CDCA) increased permeability of the BBB by activation of Rac1 followed by phosphorylation of occludin, with CDCA being more potent [54]. The disruption of BBB significantly increased with hydrophobic bile acids (DCA and CDCA) than hydrophilic bile acids (ursodeoxycholic acid, GCDCA, TCDCA) [54]. Only doubling serum total bile acid concentration was sufficient to increase BBB permeability [54]. Seiffert et al. (2004) exposed the cerebral cortex of rats to bile salts (DCA), which resulted in long-lasting extravasation of serum albumin into the brain. Although this was associated with astrocyte activation, there was no inflammatory response or marked cell loss [55]. Higher CSF levels of albumin have been reported in patients with AD [56]. Increased BBB permeability was suggested for this observation. In another *in vivo* study, focal administration of deoxycholate resulted in BBB disruption (measured using *in-vivo* MRI brain imaging) leading to neuronal loss and deterioration of animals' motor functions [57]. Previous studies have found increased permeability of the BBB in patients with AD compared to age-matched control subjects [58]. The disruption of BBB could lead to microhemorrhages with the release of neurotoxic hemoglobin-derived products [59]. The release of iron from hemoglobin can lead to lipid peroxidation and neuronal cell death [59]. It has been shown that only 1% oxidized DHA was sufficient to revert the protective effect of DHA and signif-

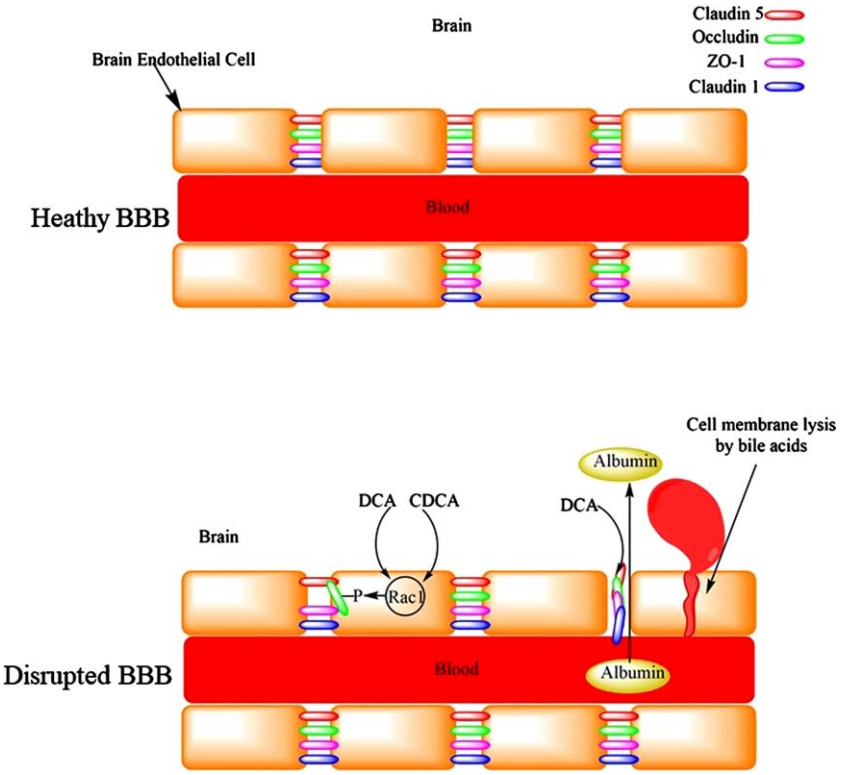

Fig. 4. The disruption of the blood-brain barrier by bile acids leading to the leakage of serum proteins like albumin into the brain or microhemorrhages.

icantly increase Aβ production [60]. It has been proposed that the disruption of the BBB would facilitate the migration of inflammatory molecules into the brain leading to microglia activation and neuroinflammation [61]. Conjugated bile acids such as TCA activated Sphingosine 1 Phosphate Receptor 2 receptor, [62] which released CCL2 from neurons, leading to activated microglia [63], and neurological decline [64]. Furthermore, inflammation would increase plasmin system inhibitors, resulting in reduced plasmin activity, which leads to impaired Aβ degradation in the brain and accumulation of Aβ deposits in AD patients [65]. The plasmin system is a set of proteases and inhibitors in the brain expressed by neurons. This system contains urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA), which convert plasminogen to plasmin (active protease). In this system tPA and uPA are inhibited by both plasminogen inhibitor-1 (PAI-1) and plasminogen inhibitor-2 (PAI-2) [66, 67], with alpha-2-antiplasmin inhibiting plasmin [66]. Interestingly, PAI-1 levels increased (not significantly) in the CSF of patients with AD compared to control subjects [67]. Therefore, the accumulation of Aβ would be expected to increase in the brains of patients with AD (increased PAI-1 levels).

Hydrophobic bile acids damage other epithelial barriers. DCA is a potent bile acid in decreasing the tight junctions of human bronchial epithelial cells at relatively mild acidic conditions (pH = 6) compared to TCA and glycocholic acid. This could be one of the reasons for pneumonia complications in AD patients [68].

POSSIBLE INCREASED C-27 BILE ACIDS LEVELS IN PLASMA OF PATIENTS WITH AD

The downregulation of D-BP enzyme in the liver of patients with AD may suggest increased levels of C27 bile acids in the serum or liver (Fig. 5), as, C27 bile acids (derivatives of 3α, 7α, 12α, 24 tetrahydroxy-5β-cholestanoic acid) account for 74% of total bile acid in the serum of patients with D-BP deficiency [69]. C27 bile acids enhance mitochondrial ROS production by inhibiting respiratory chain [70]. However, the plasma levels of 3β-hydroxy-5-cholestenoic acid (C27 bile acid) ranged

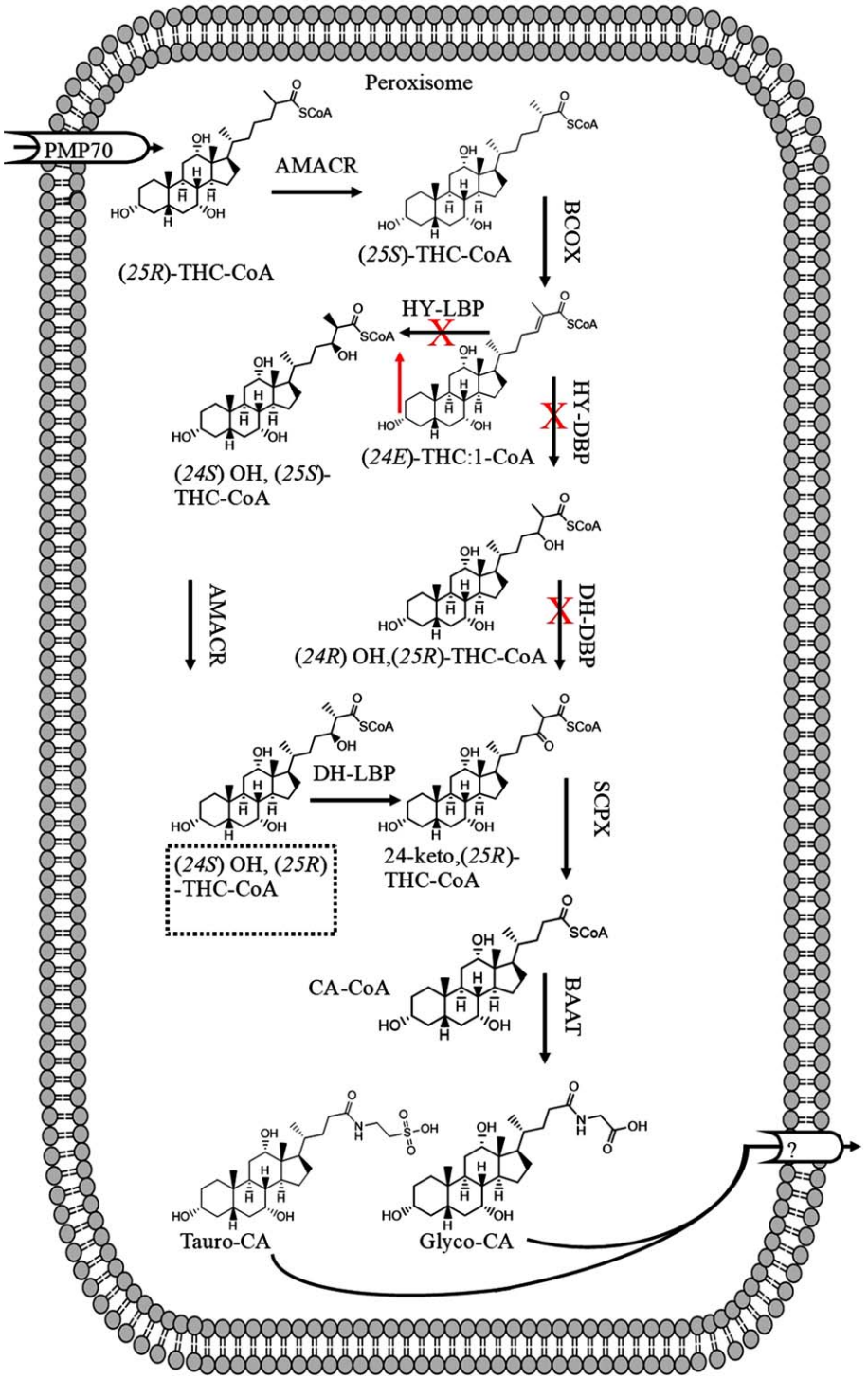

Fig. 5. The suggested disruption of bile acid synthesis in patients with Alzheimer's disease due to downregulation of HY-DBP and DH-DBP leading to accumulation of C-27 bile acids such as (*24E*) THC:1-CoA.

from 41 ng/ml to 134 ng/ml in 11 healthy subjects [71]. The total median levels of serum or plasma C27 bile acids (3β-hydroxy-5-cholestenoic acid, 3β,7α-dihydroxy-5-cholestenoic acid, 7α-hydroxy-3-oxo-4-cholestenoic acid), 172 ng/ml, was greater than median of C24 unconjugated bile acids

(146 ng/ml) in healthy subjects [72]. Certain meals (oat bran) increased serum levels of C4 (7α-hydroxy-4-cholesten-3-one) [73]. C27 bile acids in human serum originate from 7α-hydroxy-cholesterol [74]. The serum levels of C4 also increased in patients with hypercholesterolemia and treated with cholestyramine from 12 ng/ml (heathy subjects) [72, 75] to 174 ng/ml. Gälman et al. (2003) showed that treatment with cholestyramine increased rat liver 7α-hyroxylase activity leading to increased serum levels of C4 [75], and potentially serum C27 bile acid levels [76]. Furthermore, increased liver 7α-hyroxylase activity would also increase serum 7α-hydroxycholesterol levels [76]. These observations indicate that serum levels of C27 bile acids may increase due to diet, treatment, GI or hepatic diseases, and not necessarily due to downregulation of D-BP enzyme in the liver.

On the other hand, serum levels of C4 (and potentially C27 bile acid levels) decreased in patients with acute myelogenous leukemia or total parenteral nutrition [77, 78]. The levels of 7α-hydroxylase also significantly increased in patients treated with cholestyramine, but decreased in patients treated with chenodeoxycholic acid (both at the doses of 15 mg/kg) [79]. The mRNA of 7α-hydroxylase cholesterol increased in cholestatic patients, despite a reduction in 7α-cholesterol hydroxylation and bile acid synthesis [80]. These observations suggest that in certain liver diseases the serum levels of C27 bile acid and LCA may increase (derived from CDCA) due to increased serum levels of C4 [72, 81]. As CDCA is the precursor of LCA [82, 83] therefore, in AD patients when blood levels of CDCA are high, blood LCA levels tend to be high [39, 44]. As the prevalence of colorectal cancer decreases in patient with AD [84], then it may be suggested increased serum LCA levels (or potentially C4) would be transient.

On the other hand, when the serum levels of bile acids decrease in patients with AD [42], this could suggest reduction of bile acid synthesis by the liver. Then, in anyway, there would be a change in the existing balance within the intestinal microbiome of patients with AD, which is called dysbiosis. Reduced bile acid synthesis by the liver increases the small intestinal bacterial growth [85, 86], which leads to increased serum TNFα and systemic inflammation [87]. Due to proinflammatory mediators, the production of bile acids by the neutral pathway decreases and then the acid pathway produces more C4 and hence more CDCA [72]. Increased levels of CDCA increases intestinal *Enterobacteriaceae,* which are pathogenic [88]. The decreased levels of bile acids including CDCA in AD patients [42] would decrease secondary bile acids levels (including LCA) in the intestine produced by *clostridum* [89], which again leads to outgrowth of *Enterobacteriaceae* [89]. Then, the intestinal altered microbiome will lead to the formation of toxic bile acids such as DCA [90], which would damage the BBB and build up in the brain [42].

The levels of C4 are in the range of 0-15 ng/ml in healthy volunteers only for 49% of subjects. Although levels of C4 > 60 ng/ml are considered abnormal, 7% of healthy subjects fall in this range [91]. Therefore, the serum levels of C27 bile acids may be much higher in some healthy subjects compared to the others, yet with no obvious clinical symptoms.

In summary, although C27 bile acid levels may increase in the serum or liver of the patients with AD, the large variations in healthy subjects may prevent clearly seeing the increased C27 bile acids in these patients.

BILE ACIDS ENTER NEURONS VIA BILE ACID TRANSPORTERS

The solute carrier family 10 members 1 (SLC10A1; NTCP) and 2 (SLC10A2; ABST) transporter protein belong to the solute carrier super family with over 450 members [92, 93]. SLC10A1 and SLC10A2 carry bile acids. SLC10A4 is another member that is expressed in the brain [94–96]. It is located in the synaptic vesicles as either vesicular acetylcholinesterase or vesicular monoamine transporter 2 [92]. It has been proposed that SCL10A4 contributes to loading the vesicles in neurons with dopamine, which is released in synaptic clefts [92]. SCL10A4 normally has no bile acid transporter activity, but it can become an active bile acid transporter with the help of thrombin, which cleaves the N-terminus section of SLC10A4 protein (Fig. 6) [97]. Then SLC10A4 can transport LCA and DCA [97]. As thrombin levels are elevated in the brain of patients with AD [98], then this could be a mechanism that LCA finds its way into the neurons. When bile acids are in neurons, they exert their effects through a number of nuclear receptors, including farnesoid X receptor [99], pregnane X receptor [100], vitamin D receptor [101], and estrogen receptor α (discussed below). Furthermore, it has been shown that in cholestatic liver injury the serum bile acid lev-

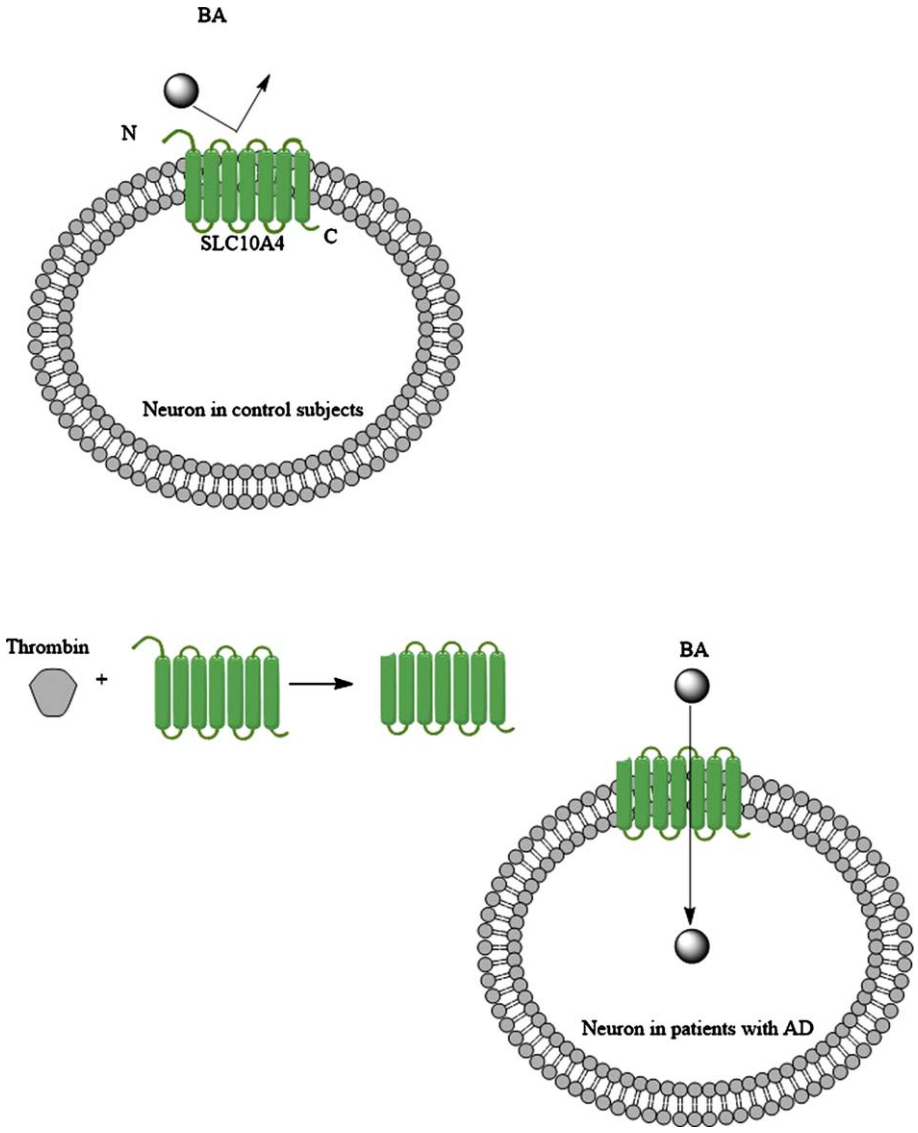

Fig. 6. Under normal condition bile acids (BAs) cannot utilize bile acid transporter like SLC104A to enter neurons, but when brain levels of thrombin increase in the patients with AD (for example due to the BBB disruption), then the SLC104A is modified, and this time it carries BAs into the neurons.

els increase. Bile acids both uncharged and negatively charged found their ways into neurons via SLC10A2 bile acid transporters, which is expressed in the rat hypothalamus; and suppressed the hypothalamic-pituitary-adrenal axis (HPA) [102]. It should be noted that SLC10A2 is not expressed in the human brain. HPA dysfunction has been reported in a substantial portion of patients with AD [103]. HPA axis dysfunction was correlated to the severity of dementia in patients with AD [103]. On the other hand, by increasing the severity of AD, depletion of SLC10A4 expression was noted in the transentorhinal cortex and hypothalamus of patients with AD [96]. Keitel et al. (2010) showed the expression of TGR5 (a membrane-bound bile acid receptor) on astrocytes and neurons [104], and the activation of TGR5 may promote the migration of bile acid transporter (ASBT) into the cell membrane [105]. However, the expression of TGR5 decreases in the brain by the elevation of serum bile acid levels [104]. This could be a further support for HPA dysfunction in AD patients in the short term, but not longitudinally [103].

THE INTERACTION OF BILE ACIDS WITH FARNESOID X RECEPTOR IN THE BRAIN

Farnesoid X receptor (FXR) protein expression was identified in the human brain abundantly [106]. In conditions when the liver is damaged, serum bile acids are known to increase, possibly due to the release of bile acid content from damaged hepatocytes [107]. When serum bile acids levels increase (for example in cholestasis), brain bile acid levels also increase [108] and toxic bile acids such as LCA tend to accumulate in the brain [108, 109]. This may also result in change of the brain bile acid profile, reduction of good bile acid levels such as CA and CDCA [109]. CDCA is the highest activator of FXR (346 folds), but LCA is the lowest FXR activator causing only 100 folds increase at 100 μM [110]. Increased bile acid levels in the brain activated FXR signaling, which downregulated the expression of brain Cyp46A1 (catalyzes the synthesis of 24(S)-hydroxycholesterol) in mice (Fig. 7) [111].

The expression of retinoid xenobiotic receptor (RXR) is reported in hippocampal neurons [112]. Therefore, RXR may heterodimerize with FXR in downregulation of Cyp46A1 (Fig. 7), as it does in the intestine [90]. This could be an explanation for the reduction of good bile acids in the brains of bile duct ligated rats [109]. In addition, the activation of FXR in the brain contributed to neurological decline [99]. Similar to the liver, activation of FXR by GW4064 increased the expression of small heterodimer partner (SHP) protein in brain primary cultured neurons [106]. More importantly, accumulation of bile acids in the brain led to neural cholesterol accumulation and neurological decline such as escape response and presence of server ataxia [111]. Bile acids bind to FXR and this prevents transactivation of LXRα, which is a positive regulator of cholesterol degradation, and LXRα is also expressed in the brain [113]. This means that activating FXR leads to accumulation of cholesterol [110]. In fact, increased total serum cholesterol levels have been observed in patients with non-cirrhotic, non-alcoholic steatohepatitis, when they were treated with 6α-ethyl CDCA (INT-747, [obeticholicacid]) [114]. This is a selective FXR agonist with 100 folds greater efficacy than CDCA [115]. Bile acids (CDCA, DCA, LCA) did not activate FXR significantly at concentrations less than 10 μM [110]. The serum total bile acid levels can increase to 71 μM in patients with liver disease compared to control subjects who have 9 μM total serum

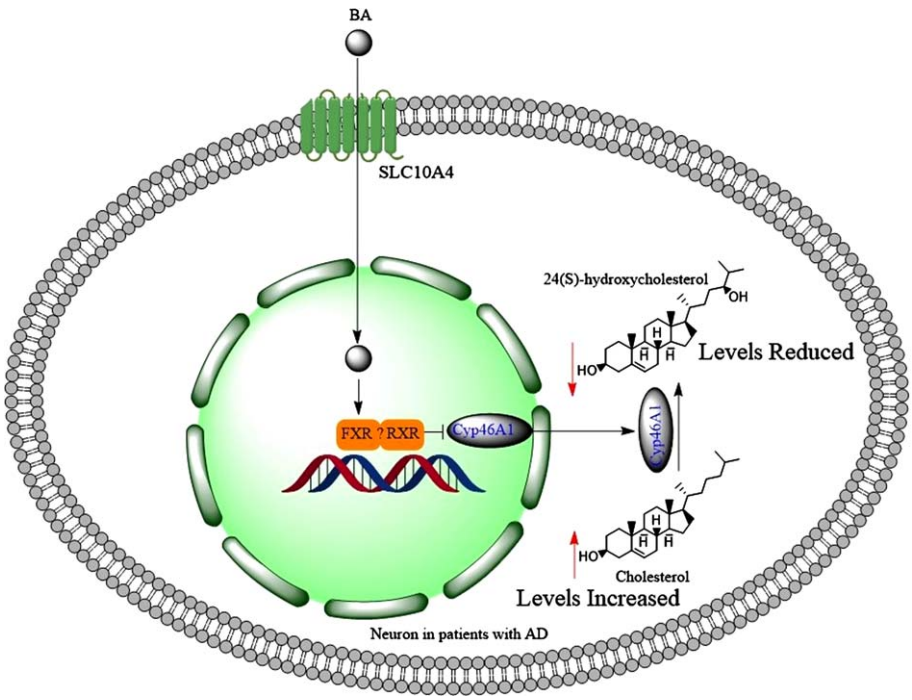

Fig. 7. When bile acids find their ways into the neurons, then they activate FXR and possibly RXR, which collectively reduce the expression of Cyp46A1 leading to accumulation of cholesterol and reduced brain levels of 24(*S*)-hydroxycholesterol.

bile acid level [116]. Therefore, significant activation of FXR can occur in the brain with serum bile acid levels under pathological conditions. These observations may explain increased levels of cholesteryl esters in the brains of patients with AD [117] through the activation of FXR signaling pathway in the brain. Reducing cortical bile acid levels alleviated accumulation of cholesterol in the brain [111]. Accumulation of cholesterol in the brain promotes the aggregation of $A\beta_{1\text{-}42}$ [118], which increases the risk of AD.

There is an involvement of phospholipase A2 (PLA2) in AD (its levels either increase or decrease), and a recent study showed that activation of FXR by CDCA at 50 μM concentration decreased phospholipase 2 G12B (PLA2G12B) expression in HepG2 (hepatoma cell line) cells [119]. Therefore, there is a possibility that part of alterations of PLA2 levels in the brains of patients with AD may originate from high toxic bile acid levels in the brain that activate FXR signaling pathway.

Activation of FXR reduced hepatic inflammation (through inhibition of NF-κB) [120]. NF-kβ inhibition also prevented the expression of proinflammatory cytokines from CNS-resident cells [121]. Furthermore, clinical aspects of experimental autoimmune encephalomyelitis (EAE) mice were significantly improved either by the inhibition of NF-kβ [122] or administration of obeticholic acid (a strong FXR agonist at the dose of 5 mg/kg) [123]. These suggest that activation of FXR would reduce neuroinflammation, a condition that has been observed in patients with AD [124]. However, toxic bile acids may contraindicate this benefit by promoting Aβ accumulation in the brain. On the other hand, NF-kβ can be activated by phosphatidylinositol 3-kinase through TNFR2 (TNF receptor 2), which promoted neuronal survival [125]. Therefore, inhibition of NF-kβ byFXR signaling could compromise neuronal survival in AD.

HYDROPHOBIC BILE ACIDS BLOCK NMDA RECEPTORS IN THE HIPPOCAMPAL NEURONS

N-methyl-D-aspartate receptors (NMDARs) play major roles in learning and memory [126–128]. NMDARs are capable of converting specific patterns of neuronal activity into long-term changes in synaptic structure (synaptic plasticity) leading to cognitive functions and learning [129]. NMDARs in the granule cells (GCs) of the dentate gyrus play a crucial role in the process of pattern separation [130]. Furthermore, new neurons are continuously generated in the hippocampus of the adult mammalian brain [131], and GluN2B containing NMDARs promote synaptic activation in adult born GCs of the olfactory bulb that integrate into circuits with high and correlated synaptic activity [132]. Two regions-the olfactory bulb (SVZ) and the dentate gyrus of the hippocampus-(SGZ)- receive and integrate new born neurons throughout the adult life [133]. NMDARs help new neurons to survive and integrate into circuits [131]. Work on rodent hippocampus has shown that NMDAR mediated plasticity (which is essential for the formation of the memory) [134] and NMDARs are crucial for the formation of temporal memory in the CA1 of the hippocampus [134]. Computational studies suggest that dentate gyrus and its connections to CA3 are responsible for pattern separation [135]. If the above pathway does not work, then the connections of CA3 to CA1 should complete the pattern [135]. Pattern completion and pattern separation complement each other. If one weakens, the other one strengthens [135]. There is a proper information encoding, normal pattern completion rate, and pattern recognition in patients with aMCI, but these are forgotten rapidly [135]. In contrast, there is a slow and even incomplete pattern recognition in patients with AD. These suggest that patients with AD have extensive hippocampal and parahippocamal damage, and therefore they cannot encode the information properly [135].

Both under-excitation (hypofunction) and excessive excitation of NMDARs are thought to play roles in AD [126, 127]. NMDAR antagonists impair spatial learning in rats [136] and nonhuman primates [137]. On the other hand, excessive activation of NMDARs (GluN2B containing NMDARs mediated by Aβ) results in deficit uptake of glutamate by excitatory amino acid transporters leading to impaired long term potentiation [126]. Although memantine is an NMDAR antagonist, memantine exerts its therapeutic efficacy by attenuating Aβ-induced tau-phosphorylation and associated signaling mechanisms [138], and it should be noted that memantine is a low-affinity NMDAR antagonist. Strong NMDAR antagonists such as phencyclidine can produce psychotomimetic effects in humans. Hydrophobic bile acids are able to inhibit NMDARs [139]. The affinity of bile acids to albumin determines the potency of a bile acid in blocking NMDARs [139] with LCA being the most potent molecule [140]. Blockade of NMDARs by bile acids would be expected,

as pregnanolone sulfate is a non-competitive antagonist of NMDARs and structurally is similar to sulfated LCA. NMDARs are crucial for the formation of temporal memory in the CA1 [134], and the NR2A subunits of NMDARs are responsible for the retrieval of memory [141]. The blocking of NMDARs results in apoptosis of neuroblasts and this decreases the number of newly generated neurons [142] and hence formation of new memories [143]. The restoring of spine densities in the dentate gyrus rescued loss of long-term memories in mouse models of AD [144] and NMDARs are required for the formation of spines [145]. Furthermore, the blockade of NMDAR in retrosplenial cortex disrupts retrieval of remote and recent information [141], decreases synthesis of 17β estradiol in the brain [146], inactivates benefits of E2 via Rap/AF-6/ERK1/2 signaling pathway [147], reduces neural connectivity [148] (dendritic complexity, spine density and morphology), prevents persistent and maturation of newly formed synapses [149], and contributes to NMDAR hypofunction (which has been seen in AD) [150]. Hydrophobic bile acids reduce the levels of 24-OHC in the brain [151], which is a strong activator of NMDARs [152]. As presynaptic NMDARs (PreNMDARs) would enhance transmitter release in part via protein kinase C signaling [153] and subsequent glutamate release in CA3-CA1 circuit [154], then blockade of preNMDARs by toxic bile acids would further contribute to the dysfunction of remaining NMDARs in AD [155]. Blockade of NMDAR with toxic bile acids may lead to psychotic symptoms that has been observed in 50% of patients with AD [156].

Activation of NMDARs leads to the activation of mitogen-activated protein kinase (MAPK) and extracellular receptor kinase-1and 2 (ERK1/2), inducing phosphorylation of cAMP responsive element binding protein (CREB) and increasing expression of brain-derived neurotrophic factor (BDNF). This contributes to memory formation and cognitive function (Fig. 8) [157–159].

HYDROPHOBIC BILE ACIDS COULD IMPAIR THE ROLES OF E2 IN THE BRAIN

17β estradiol (E2) is a steroid hormone with molecular weight of 272 Da which is biosynthesized from cholesterol involving aromatase [160]. Hippocampal

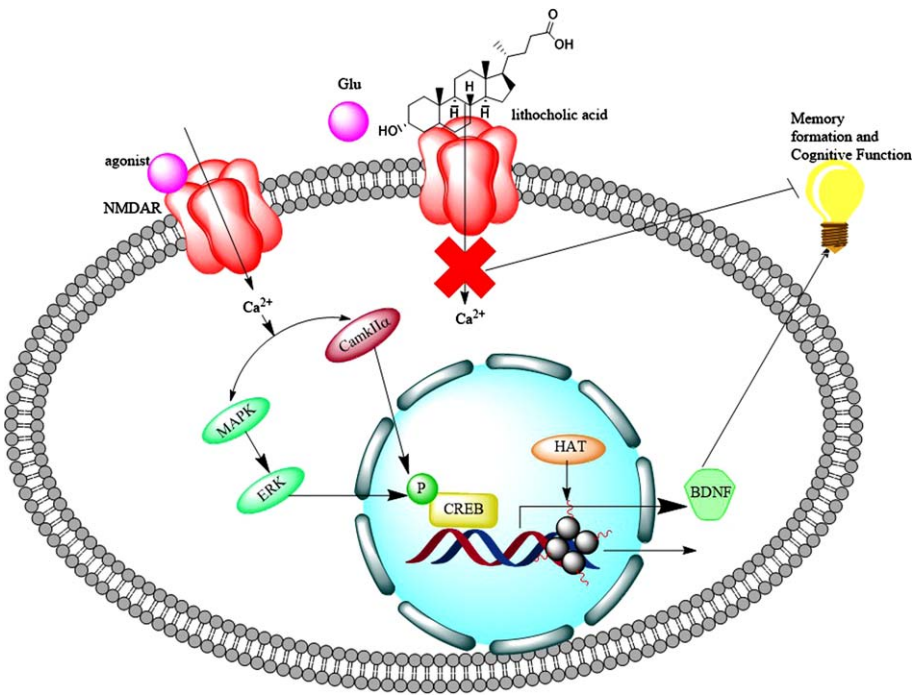

Fig. 8. Hydrophobic bile acids such as lithocholic acid block the activation of NMDARs by agonists such as glutamate (Glu); and prevent flux of Ca^{2+} into neurons leading to downregulation of BDNF, which contributes to the formation of memory and cognitive function. The flux of Ca^{2+} activates both cAMP-responsive element binding protein mitogen-activated protein kinase/extracellular regulated kinase MAPK/ERK and calcium/calmodulin-dependent protein kinases IIα (CamkIIα) [159] which results in phosphorylation of cAMP-responsive element binding protein (CREB) as well as activation of histone acetyl transferase (HAT). These lead to upregulation of BDNF.

pyramidal neurons and granule neurons of adult male rats are equipped with a complete machinery for the synthesis of E2 from endogenous cholesterol [161]. Furthermore, brain has the capacity to convert circulatory testosterone into E2 through the enzymatic action of aromatase [162]. E2 plays major roles in the brain such as reducing neuronal loss following stroke, increasing neuronal connectivity, improving cognitive performance, inducing brain BDNF synthesis [163–166], improving learning in ovariectomized (OVX) mice [167], and contributing to leaning by increasing hippocampal dendritic spines [168-170]. E2 exerts its effects in the brain through estrogen receptor α (ERα), ERβ, and GPER1 [171]. ERα facilitated cognitive functions [172], contributed to the BBB functions [173], reduced Aβ accumulation in the brain [174], reduced tau phosphorylation by phosphorylating GSK3β [175], reduced BCL-associated death promoter (BAD) levels [176], and generated optimal signals of E2 through proline-, glutamic acid-, and leucine-rich protein-1 (PELP1) [177]. Furthermore, E2 interacts with GSK3β and β-catenin in the brain via ERα, which is crucial in ERα protein stabilization and turnover [178]. E2 mediates neuroprotection [179] via activation of plasma membrane ERα [180, 181], ERβ [182], GPER1 (GPR30) [183], extranuclear ER [184], via PELP1 interaction with GSK3β [185], and inhibition of NRLP3 inflammasome pathway activation [186]. E2 also improved episodic memory [187], LTP [188], and neurogenesis [189], through NMDARs. Spine density significantly increased with initial E2 treatment followed by NMDAR activation [147].

The estrogen receptor is unique in having a glutamate (Glu-353) to accept the hydrogen bond donated by the estrogenic 3-hydroxyl group [190]. The 17β-hydroxyl group of E2 makes a single hydrogen bond with the δ nitrogen of His-524 in hERα [191]. Most of ERα ligands contain 3-hydroxyl group [192], similar to LCA. However, LCA contains 19-methyl group, which makes LCA a weak partial agonist, and could displace more potent ligands from their binding sites [193]. Our molecular modelling studies suggests that LCA can bind to ERα receptors (Fig. 9), but possibly void of physiological actions (the molecular modelling investigations are exclusive to this review paper and were performed in Autodock vina version 1.1.2 [194, 195]). This is because upon binding of LCA with ERα, the helix 12 of ERα cannot cover as a lid over the ligand-binding pocket to secure the ligand in position. Hence, having ERα occupied by less potent ligands, this would reduce the efficacy of E2 to activate desired signaling pathways. Our results may be supported by previous observations about transrepression of bile salt export pump (BSEP) mediated by E2 through ERα [196]. CDCA strongly transactivated BSEP expression and E2 repressed this activity. However, E2 efficacy was much lower in the presence of LCA [196]. Therefore, it may be interpreted that LCA reduced the efficacy of E2, through occupying

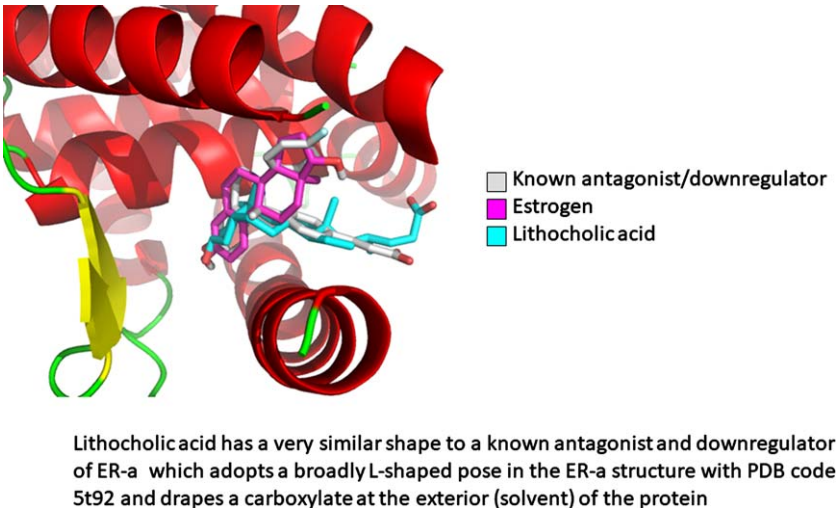

Fig. 9. The molecular modelling of the interaction of estrogen receptor-α (ERα) with lithocholic acid. Lithocholic acid has a very similar shape to a known antagonist (compound 9 in reference [398], the molecular structure of the compound is given in Supplementary Figure 3) and down regulator of ERα which adopts a broadly L-shaped pose in the ERα structure with PDB code 5t92 and drapes a carboxylate at the exterior (solvent) of the protein.

ERα and preventing E2 to fully engage with ERα. Furthermore, LCA has been considered as a ligand which has similar lipophilicity to estrogen and possibly with the ability to interact with estrogen receptors such as ERα, but devoid of metabolic activity, which estrogen is able to exert [197].

Leu-387 in ER will act like a barrier from complete fitting of LCA to the ER ligand-binding pocket. E2 acts through ERα to acutely suppress GABA release [198]. The interference of LCA with E2 roles may explain the disturbed balance of excitatory and inhibitory signaling systems observed in AD brains [199]. LCA inhibited NLRP3 inflammasome activation [200], similar to E2. LCA does not activate ERβ [201, 202]. Furthermore, LCA reduced significantly the expression of ERα in MCF-7 cells [203], and the reduction of cytoplasmic ERα expression has been reported in the brains of patients with AD compared to control subjects [204].

The overall level of ERα does not significantly change in AD brains compared to control subjects, although there is a slight decrease compared to control subjects [205]. Therefore LCA could reduce the number of accessible ERα and diminish working memory performance [206]. Furthermore, the percentage of neurons expressing ER-mRNA decreases with age after 65 years [207], and there is a relationship between MMSE and levels of wild-type nuclear fraction ERα in superior frontal cortex of patients with AD [208].

The neuroprotection exerted by IGF-I also depends on estrogen receptors [209]. Although the levels of IGF-I increase in the CSF of patients with AD [210], the neuroprotective effects of IGF-1 are not seen. This might be explained by the blockade of ERα by LCA, and IGF-I requires involvement of ERα for its roles. Furthermore, BAD levels increase in the brains of patients with AD [211], along increased activity of GSK3β [212]. In conclusion, the above evidence suggests that LCA may interfere with the vast benefits of E2 in the brain via ERα receptor.

LITHOCHOLIC ACID AND THE FUNCTIONS OF ABCA1 AND P-GLYCOPROTEIN IN REDUCING BRAIN Aβ LEVELS

ATP-binding cassette (ABC) transporters utilize ATP to transport their substrates across membranes. ABCA1 (also known as CERP) is a member of ABC transporters that is expressed on the human BBB endothelial cells [213–215] (both luminal and abluminal sides in porcine [216]) and human astrocytes, pericytes and microglia [217]. There is a direct correlation between ABCA1 expression and lipidation of apoE in the CNS, indicating that ABCA1 transports cholesterol on to apoE [218] and interestingly, ABCA1 is required for normal levels of apoE in the brain [219]. The ABCA1 indirectly eliminates $A\beta_{1\text{-}40}$ from the brain into the blood circulation through the BBB [220]. The ABCA1 of microglia cells is required for efflux of cholesterol to apoA-I, apoE2, and apoE3 [221], a major contributing pathways for regulating lipid homeostasis in the brain. As expected, the inhibition of ABCA1 resulted in the accumulation of cholesterol in the brain [222]. Furthermore, ABCA1 reduced neuroinflammation and neuronal death [223].

Lipidated apoE by ABCA1 promoted degradation of soluble Aβ both inside microglia and extracellularly [218, 224], and ABCA1 (even non-functional mutants) reduced Aβ production [225]. Astrocytes are the major source of extracellular apoE in the CNS and themselves express a high level of ABCA1, which plays a crucial role in extracellular lipidated apoE [226], facilitating degradation of soluble Aβ by insulin degrading enzyme [224], the main proteolytic degradation pathway of Aβ [227]. It should be noted that there are three major isoforms of apoE (apoE2, apoE3, apoE4), with lipidated apoE3 presenting 2-3 fold higher affinity for both $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides than apoE4 [228]. On the other hand, delipidation of both apoE3 and apoE4 decreased their affinities for Aβ peptides by 5-10-fold and abolished the isoform-specificity [228]. In fact, overexpressing ABCA1 increased lipidation of apoE in the CNS and reduced amyloid burden [229]. Conversely, the absence of ABCA1 resulted in a significant increase in $A\beta_{1\text{-}40}$ load in the brain of $ABCA1^{-/-}$ mice compared to the wild type [230]. It has been shown that increased brain ABCA1 levels by oral administration of rosiglitazone improved Aβ clearance from the brains of AD rat models [231]. ABCA1 is upregulated in AD hippocampal neurons two- to three-fold, potentially through Aβ mediated pathways [232]. The increased levels of ABCA1 in the brains of patients with AD may be a compensatory mechanism to reduce the brain Aβ load [233]. Overexpressing ABCA1 in APP/PS1 mice reduced brain Aβ burden [234]. It should be noted that environmental factors (such as dichlorodiphenyltrichloroethane) could increase brain Aβ levels by lowering ABCA1 expression and promoting Aβ synthesis [235].

LCA reduced the expression of ABCA1 in HepG2 cells through the activation of nuclear pregnane X receptor (PXR) [100]. In fact, PXR serves as a physiological sensor of LCA [236]. Activation of PXR increased ABCA1 expression in kidneys [237]. Activation of PXR in the brain increased the expression of P-glycoprotein (P-gp) [238] and significantly reduced brain Aβ levels in a mouse model of AD. Therefore, it would be expected that P-gp expression is increased as the result of PXR activation by LCA. At the early stage of AD, P-gp expression is upregulated in the capillaries of the brain. However, the accumulation of Aβ completely disappeared the expression of P-gp on arterioles or reduced P-gp capillary expression [239, 240]. A compensatory mechanism may be suggested to increase Aβ clearance from the brain by upregulation of P-gp [239], high serum LCA levels may also contribute to increased P-gp expression via PXR activation. Furthermore, it was found that activation of PXR (by allopregnanolone or T0901317 [241]) led to increased expression of ABCA1 in mouse brain [242]. Therefore, the increased expression of ABCA1 in the brain of patients with AD could be due to Aβ-mediated pathway, as well as increased serum LCA levels. It appears that LCA may provide some neuroprotective effects against AD. However, this may lead to increased brain Aβ-levels, and contraindicates the neuroprotective effects (Fig. 10).

TOXIC BILE ACIDS AFFECT 24-OHC AND BILE ACID PROFILES IN THE BRAIN

Cholesterol 24-hydroxylase (CYP46A1) is highly expressed in the brain and catalyzes the synthesis of 24(S)-hydroxycholesterol (24-OHC) from cholesterol (Fig. 11) [243]. CYP46A1 is not uniformly distributed in the human brain, but it has been detected in the hippocampus [244]. The formation of 24-OHC is a major pathway for removal and excretion of cholesterol from the brain [245]. The net flux of 24-OHC is 6.4 mg/day to the blood circulation for human brain [246]. The brain contains about 80% of 24-OHC in the body [247], therefore, 24-OHC is also called cerebrosterol [248]. The net flux of 24-OHC remains age independent for adults older than 20 years [247]. As well as net flux of 24-OHC to the blood circulation and conversion to bile acids by the liver, brain has all the enzymes to biosynthesis for CDCA from 24-OHC [249].

24-OHC induced transcription and protein synthesis in a dose dependent manner in astrocytes, but not in SH-SY5Y cells (neuronal origin) cells [250]. This led to increased efflux of cholesterol via apo-E and apoA-I [250]. Following a traumatic brain injury, there is an increased efflux of cholesterol from damaged cell membrane to the brain environment; and as a result the activity of CYP46A1 increases to convert excess insoluble cholesterol to soluble form of 24-OHC [251]. As well as trauma, inflammation and necrosis are associated with increased cholesterol production in the brain, which leads to increased amounts of 24-OHC in the brain [252]. 24-OHC is an LXR agonist, and consequently can increase the expression of ABCA1 [253], contributing to reduction of AβPP cleavage and Aβ production, i.e., potentially reducing the risk of developing AD [254]. Furthermore, 24-OHC has positive modulatory effects on NMDARs [152] promoting LTP and cognitive functions [152]. In fact, 24-OHC is a very potent, direct, and selective positive allosteric modulator of NMDARs with a mechanism that does not overlap with other allosteric modulators such cholesterol or 27-hydroxycholesterol [152]. Finally, under normal conditions, 24-OHC favors the processing of AβPP to the non-amyloidogenic pathway [255].

The serum levels of 24-OHC is a balance between the brain production of 24-OHC and its metabolism by the liver [256]. Then higher serum 24-OHC level would be expected in patients with liver disease compared to controlled subjects [257]. Serum levels of 24-OHC varied between 1.7-18 μmol/L in patients with severe cholestatic liver disease (equivalent to 685 ng/mL-7,236 ng/mL, Mw = 402 g for 24-OHC) [258], while normal 24-OHC levels are in the range of 30-80 ng/mL [259]. Therefore, increased serum levels of 24-OHC in patients with liver disease could be due to the inability of the liver to convert it to bile acids such as CDCA [260]. On the other hand, the plasma or serum levels of 24-OHC were significantly lower in patients with AD (20-50 ng/mL) than matched control subjects (30-80 ng/mL) [259, 261]. The reduced plasma levels of 24-OHC could suggest the loss of CYP46A1 in the brain [262]. Then expectedly, variation in *CYP46A1* gene may act as a risk factor for AD through influence on cholesterol metabolism in the brain [263]. It was found that the number of neurons and astrocytes expressing CYP46A1 decreased in the cortex of patients with AD compared to control subjects [264, 265]. Interestingly, the remaining CYP46A1 enzymes were accumulated around Aβ plaques [264].

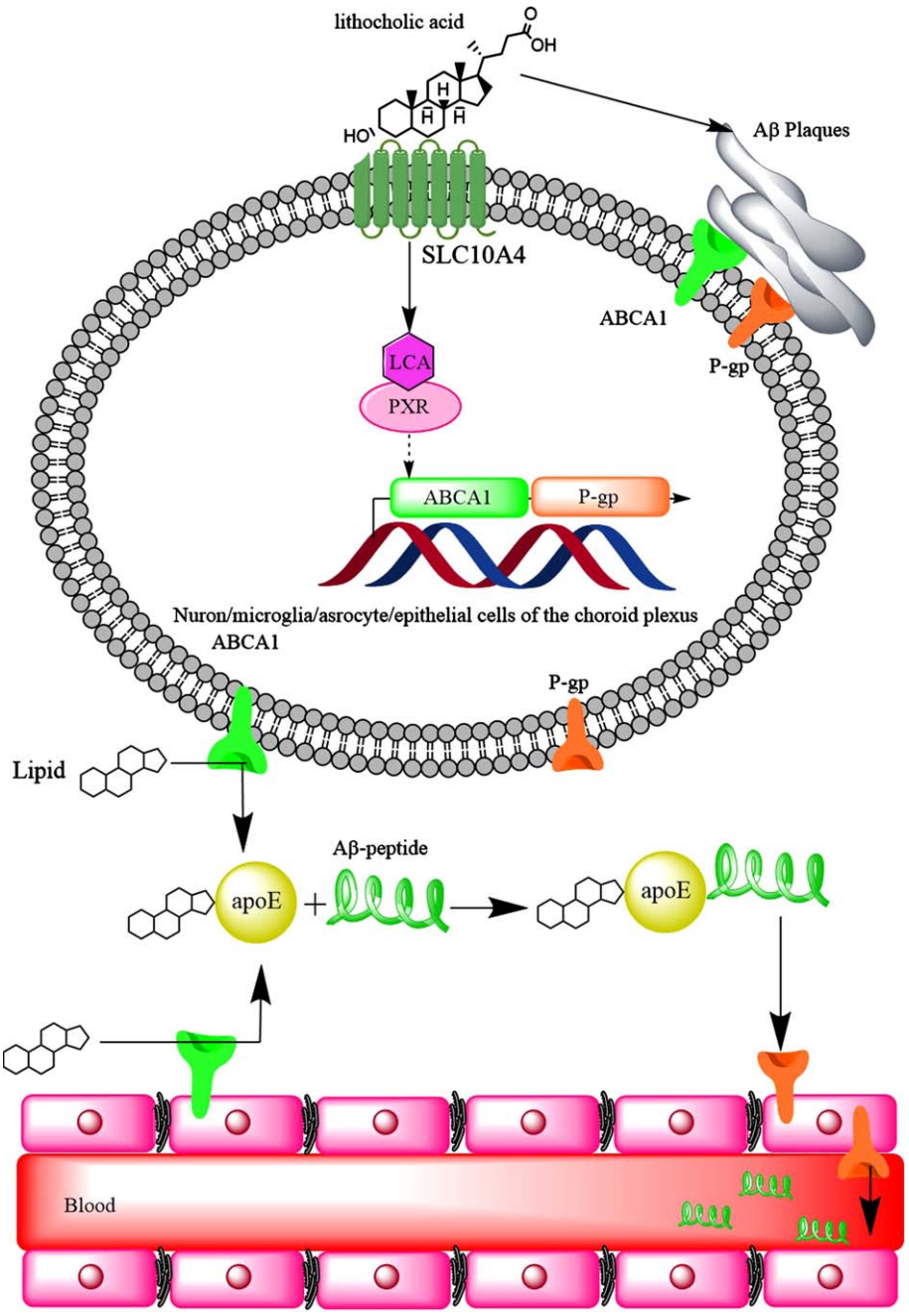

Fig. 10. The hydrophobic bile acids such as lithocholic acid increase the expression of ABCA1 transporter and P-gp. Although upregulation of these proteins should lead to better clearance of Aβ plaques from the brain of AD patients, the hydrophobic bile acids promote the formation of Aβ plaques which themselves make ABCA1 and P-gp out of function.

Can elevated serum bile acid levels suppress CYP46A1 expression in the brain, and hence reduce 24-OHC levels in the brain? *In vivo* studies showed that mice with acute liver failure, which was induced by azoxymethane, developed increased serum bile acid levels [266]. Toxic bile acids such as LCA and DCA reduced the synthesis of 24-OHC and expression of CYP46A1 in the brain via activation of FXR and SHP [151] (Fig. 11). Therefore, decreased expression of CYP46A1 in the brains of patients with AD could be due to the increased serum bile acid levels. This is in line with reports that CSF [267]

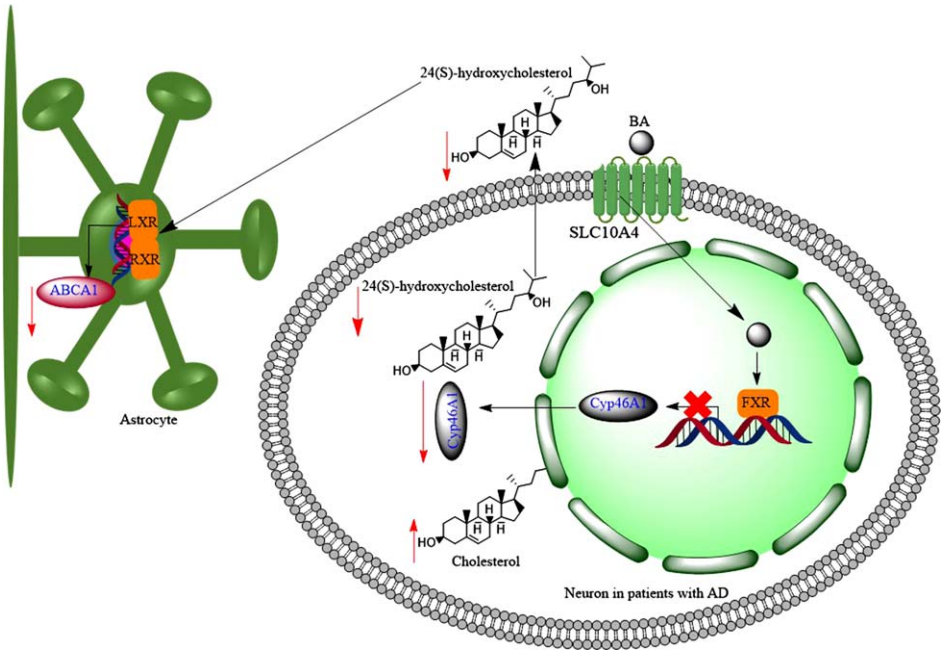

Fig. 11. Hydrophobic bile acids (BAs) reduce brain levels of 24(S)-hydroxycholesterol, through downregulation of Cyp46A1. 24(S)-hydroxycholesterol increases the expression of ABCA1 transporter in astrocytes through activation of LXR-RXR signalling pathway, then reduction of 24(S)-hydroxycholesterol in the brain may contribute to downregulation of ABCA1 in astrocytes.

and brain levels of 24-OHC decrease as the severity of AD progresses [268]. Although, concentrations of 24-OHC in the CSF were either significantly higher in patients with AD (2.28 ± 0.73 ng/mL at the early stages, or 2.03 ± 0.36 ng/mL at the later stage) [269], or slightly higher than control subjects [270], higher early CSF 24-OHC levels in patients with AD could be due to the increased neurodegeneration [248]. Normal CSF 24-OHC concentration is 1.5 ng/mL [249]. Furthermore, it was confirmed that the reduction of 24-OHC was accompanied by the reduction in the expression of CYP46A1 in postmortem human AD brains [268]. Although, there has not been a report on the CSF levels of LCA in patients with AD and at the same time for 24-OHC levels, the CSF levels of GLCA and TLCA increased in patients with AD [271].

Therefore, it may be speculated that at the early stages of the AD, increased rate of neuron breakdown produces large amounts of 24-OHC in the brain and as a result CSF levels of 24-OHC increase. However, the negative feedback of serum high secondary bile acid levels such as LCA reduce the expression of CYP46A1 in the brain and hence CSF levels of 24-OHC decrease at the later stages of the AD.

THE INTERACTION OF HYDROPHOBIC BILE ACIDS WITH TGR5 RECEPTORS

Takeda G protein receptor 5 (TGR5) is a cell-surface G protein coupled receptor, which is responsive to bile acids [272]. TGR5 is expressed in gastrointestinal tract, on immune cell membranes, neurons and astrocytes [273]. TGR5 plasma membrane localization and responsiveness to extracellular ligands depends on a long (>9 residues) α-helical stretch at the C terminus [274]. Bile acids activate TGR5 with different potencies and in the rank order of LCA > DCA>CDCA>CA [275]. Among all bile acids, TLCA is the most potent agonist of TGR5 [276]. TLCA has a 3-hydroxyl group which forms hydrogen bond with Y240 of TGR5. This is essential for the high activity of TLCA [277]. Bile acids interact in different modes with TGR5 leading to different cellular responses [277]. For example, tauroursodeoxycholic acid (TUDCA) with a 7-hydroxyl (as well as 3-hydroxyl group) forms a hydrogen bond with $N93^{3.33}$ of TGR5 [277]. While LCA with 3-hydroxyl group (lack of 7-hydroxyl group) forms a hydrogen bond with $Y89^{3.29}$ or $N93^{3.33}$ (depending on the head-to-tail, or tail-to-head pose in the inter-

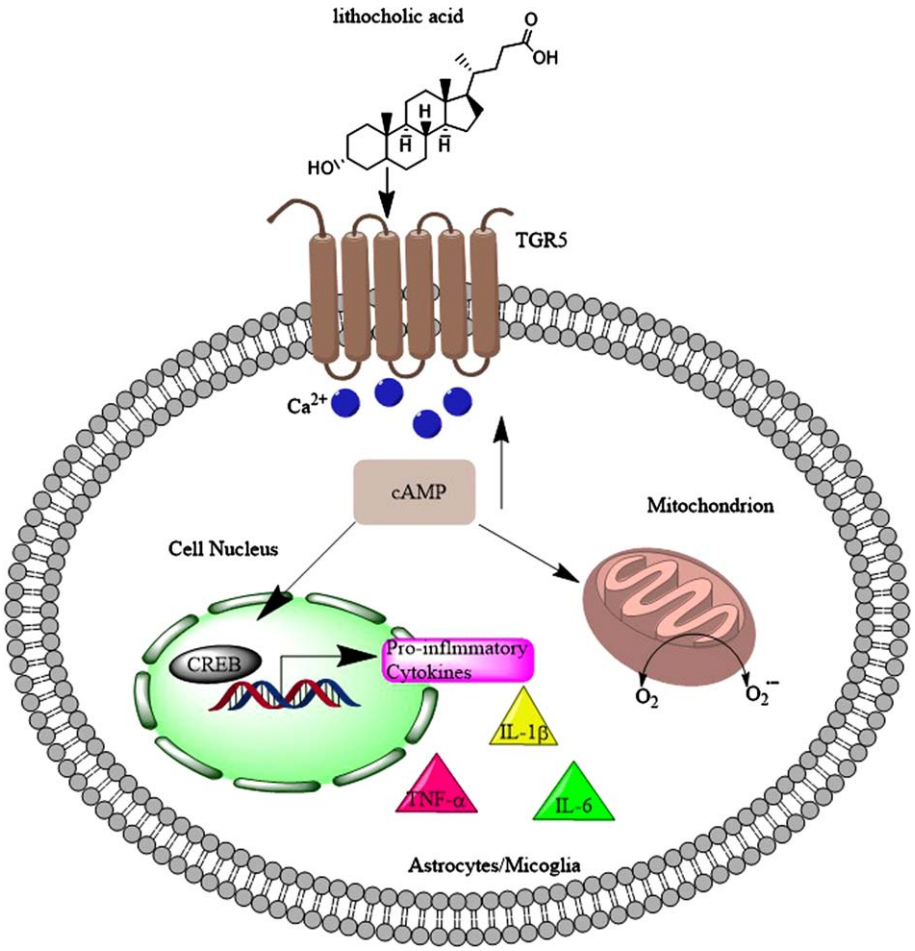

Fig. 12. Toxic bile acids such as lithocholic acid activate TGR5 in microglia and astrocytes which leads to the flux of Ca^{2+} into these cells. As a result, the levels of cyclic adenosine monophosphate (cAMP) increase, which not only increases the reactive oxygen species (ROS) levels, but also leads to activation of CREB and production of pro-inflammatory cytokines (TNFα, IL-1β, and IL-6).

action site) of TGR5 [278]. Alternatively, toxic bile acids may transactivate other receptors, such as DC (deoxycholate) transactivating epidermal growth factor receptor (EGFR)-ERK1/2 signaling pathway via TGR5 [279], leading to different responses.

What are physiological roles of TGR5 in the brain?

There is a body of evidence that activation of TGR5 by ligands (including bile acids) would reduce inflammatory, induce anti-inflammatory markers [280–286], reduced the progression of the disease in amyotrophic lateral sclerosis [287], and significantly decreased the levels of TNF-α, IL-1β and IL-6, following injection of $A\beta_{1\text{-}42}$ into the brain [288]. On the other hand, TGR5 stimulation by 5β-pregnan-3α-ol-20-one and 5β-pregnan-3α-17α-21-triol-20-one (both structurally similar to LCA with 3-hydroxyl groups) are coupled to the elevation of intracellular Ca^{2+} and the generation of reactive oxygen species (Fig. 12) in astrocytes and neurons [104]. Furthermore, microglia cell lines showed slightly reduced inflammation following TGR5 activation by betulinic acid (a 3-hydroxyl group bile acid, but with two methyl groups at position 4, Supplementary Figure 3) in the range of 10 μM to 1 mM [286].

Bile acids may promote neurological functions through TGR5 activation. TUDCA improved neurological functions through TGR5/SIRT3 signaling pathway after subarachnoid hemorrhage (SAH) [289]. Also, INT-777 (Supplementary Figure 3) administration significantly decreased NLRP3-ASC inflammasome activation in microglia, reduced brain edema and neuroinflammation, leading to improved short-term neurobehavioral functions at 24 h after

SAH [290], and significantly improved $A\beta_{1-42}$-induced cognitive impairment (administered i.c.v.) [288]. Activating TGR5 by INT-777 reduced BBB permeability and improved neurological functions through the BRCA1/Sirt1 signaling pathway after middle cerebral artery occlusion [291]. The activation of TGR5 with INT-777 significantly improved the short-term and long-term neurological deficits, accompanied by reducing the oxidative stress and neuronal apoptosis at 24 h after SAH [292]. Oleanolic acid is a TGR5 agonist [293] and administration of oleanolic acid to EAE mice reduced the severity of the disease by reduction of the BBB leakage and lowering infiltration of inflammatory cells into the CNS [294]. TGR5 mRNA was downregulated in the cerebral cortex of cirrhotic patients dying with hepatic encephalopathy when compared with the brains from non-cirrhotic control subjects [104], perhaps due to increased serum bile acid levels in these patients [295]. Also, western diet increased brain levels of DCA, which was followed by reduced expression of TGR5 in the brain [296]. In addition, ammonia [104] downregulated the expression of TGR5 in the brain, and the brain levels of ammonia significantly increase in patients with AD [297]. Therefore, toxic metabolites such as bile acids may prevents the benefits of other bile acids by reducing TGR5 expression.

Does TGR5 play a role in AD?

$A\beta_{1-42}$ in the brain downregulated the expression of TGR5 in the hippocampus [288]. Bile acids activate TGR5 on sensory nerves, stimulating the release of neuropeptides in the spinal cord that transmit itch and analgesia [298]. Patients with AD [299] or dementia [300] experience pruritus.

All the above observations lead to the conclusion that certain TGR5 signaling by bile acids such as TUDCA and INT-777 is neuroprotective and diminishes inflammation. However, TGR5 expression is downregulated in the brains of patients with AD, and toxic bile acids may contribution.

HYDROPHOBIC BILE ACIDS MAY IMPAIR SONIC HEDGEHOG SIGNALING PATHWAY IN THE BRAIN

Sonic hedgehog (SHH) is a mammalian glycoprotein involved in both during embryonic development and also post-embryonic tissue regeneration and repair [301]. The SHH expression increases by age in the cortex, hippocampus, and cerebellum regions of rat brains [302]. In addition, SHH signaling regulates the self-renewal of neuronal stems cell in the subventricular zone of adult brains [303] that continuously supply new neurons [304]. SHH is associated with cholesterol rich raft-like microdomains [305]. Lipid rafts represent discontinuous regions of the plasma membrane that form functional microdomains [306], which are enriched sterol, sphingolipids, and glycosylphosphatidylinositol-linked proteins (Fig. 13A) [307]. The SHH signaling pathway was reduced in 3xTgAD animals [306]. Inhibitors of γ-secretase restore SHH signaling, which are currently envisaged as tools for the cure of AD, because they lower Aβ levels [308]. The brains of AD patients show a specific down-regulation of seladin-1, a protein involved in cholesterol synthesis, and low membrane cholesterol was observed in hippocampal membranes of ApoE4 (apolipoprotein E4) AD cases [309]. As explained in the above, cholesterol is required for the activity of SHH protein. The expression of PTCH1 was almost missing in the brains of patients with AD, as well as decrease in Ptch2 [310]. Interestingly, $A\beta_{1-42}$ decreased brain Ptch-Gli1 levels, while it elevated SHH expression [310]. Increased levels of SHH expression were found in the hippocampus of APP23 mice and AD patients [310, 311]. These observations indicate that although SHH levels increase in the brains of patients with AD, the other components for full SHH signaling are not available. The early and progressive cholinergic deficiency of basal forebrain cholinergic neurons (BFCNs), characterized by a reduction in acetylcholine synthesis, substantially contributes to the gradual cognitive decline of AD patients [312]. Work by Yue et al. (2015) showed the important role of SHH in derivation of embryonic stem cells to BFCNs [312]. This could highlight that lack of functional SHH in the brain of patients with AD, which may prevent derivation of endogenous stem cells in the brain to BFCNs.

In the brain, the presence of ROS leads to autoxidation of cholesterol and production of 7-keto-cholesterol [313, 314]. CYP27A1(in the brain) [315] produces 7-keto-27-OHC from 7-keto-cholesterol, and 7-keto-27-OHC binds cysteine-rich domain of SMO activating SHH [316]. Interestingly, the levels of 7-keto-cholesterol significantly increase in the brain of patients with AD [268], suggesting activation of SHH signaling pathway in AD. However, it was shown that $A\beta_{1-42}$ interrupted canonical SHH signal transduction, leading to distorted primary cilia struc-

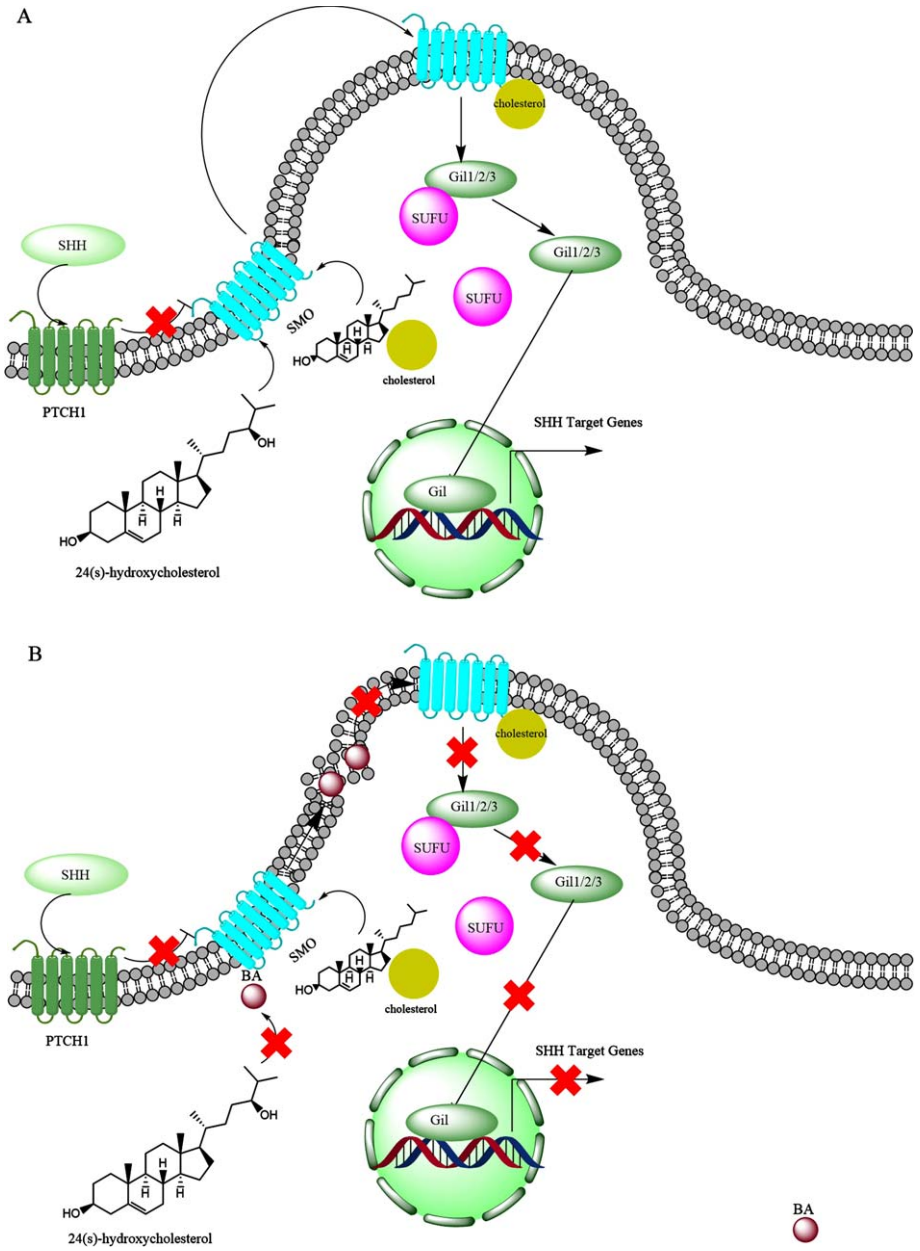

Fig. 13. The possible interference of bile acids (BAs) with the sonic hedgehog (SHH) signaling. A) Under normal condition, SHH protein interacts with PTCH1, which inhibits smoothened protein (SMO). Upon the activation by the SHH, the SMO forms a complex with oxysterols like 24(S)-hydroxycholesterol and also is conjugated with cholesterol which leads to movement in the lipid raft and activation of Gli family proteins (Gli1, Gli2, Gli3). Initially the Gli proteins are in complex with SUFU protein, but upon interaction with SMO protein, SUFU is separated from the Gli family proteins. Then the released Gli family proteins are translocated to the nucleus, where over express SHH target genes. B) BAs may interfere with SHH signaling by preventing activation of SMO by oxysterols as well as modifications of lipid rafts which may prevent activation of Gli family proteins.

ture, which are found in hippocampal neurons [317]. On the other hand, reducing SHH activity by protease nexin-1 helped improving memory functions of AD transgenic mice (APP/PA1) [318]. Meanwhile, facilitating the expression (over expressing) of SHH contributes to neurogenesis [319]. It has been suggested that the deregulation of PTCH1-Gli1 signaling in AD leads to the loss of NSCs and glial precursor cells and consequently decline in cognitive function [320].

The above controversial observations may be explained by the toxic bile acid interventions. Toxic bile acids like DCA preferentially interact with disordered membranes and makes them more disordered [321]. The hypothesis that changes in the lipid composition of rafts contribute to AD pathology has gained considerable support [306]. It was found that cortical lipid rafts are profoundly affected in the 3xTgAD mice [306]. Therefore, toxic bile acids might interfere with SHH signaling, by imposing perturbations into plasma membrane lipid rafts (Fig. 13B).

LITHOCHOLIC ACID MAY PROMOTE THE FORMATION OF Aβ PLAQUES

Amyloid formation by $A\beta_{1\text{-}40}$, $A\beta_{26\text{-}39}$, and $A\beta_{26\text{-}40}$ can be nucleated-"seeded"-, by peptides which hold the critical C-terminal residues including $A\beta_{1\text{-}42}$, $A\beta_{26\text{-}42}$, $A\beta_{26\text{-}43}$, and $A\beta_{4\text{-}42}$ [322]. The key amino acids are isoleucine and alanine [322]. However, both $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ formed filaments [323]. Importantly, $A\beta_{1\text{-}42}$ accelerated the nucleation of $A\beta_{1\text{-}40}$ [324] with Aβ trimer sizes of 20 nm [325]. $A\beta_{1\text{-}42}$ promoted Aβ deposition, but $A\beta_{1\text{-}40}$ inhibited it [326–328]. Furthermore, Aβ aggregates induced formation of more Aβ aggregates [329]. Other metabolites or compounds also can affect the formation of Aβ aggregates such as retinola [330], vitamin D binding protein (vDBP) [331], vitamin D2 (V-D2) [332], and vitamin D (vitamin D3) [333]. Our molecular modelling studies also suggested that LCA forms a complex with $A\beta_{1\text{-}40}$ similar to V-D2, hence, LCA may also promote the aggregation of $A\beta_{1\text{-}40}$. Figure 14 represents the docking performed between $A\beta_{1\text{-}40}$ taken from NMR structure with PDB code 1iyt and vitamin D2 or LCA. The docking was performed in Autodock vina version 1.1.2. and is analogous to that performed previously, the chain selected from docking was that labelled 2, which was found to resemble most closely that used previously [194, 195, 332].

Both LCA and vitamin D3 (1,25 $(OH)_2D3$) bind to vitamin D receptor, with LCA having less affinity [334]. Therefore, LCA may interfere with the biological benefits of vitamin D3 and could promote the aggregation of Aβ. This could have further consequences. In more details, two binding sites have been identified for vDBP, one for vitamin D and one for actin [335]. vDBP may also bind bile acids, because there is more ligand structure flexibility for

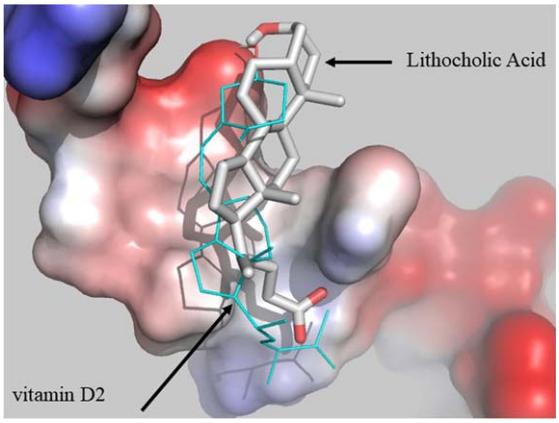

Fig. 14. Molecular modelling simulation of docking between LCA (grey sticks), vitamin D2 (cyan lines) and $A\beta_{1\text{-}40}$, demonstrating possible promotion of Aβ plaque formation by LCA.

vDBP compared to VDR [336]. Furthermore, vitamin D2 metabolites bind human vDBP with less affinity than vitamin D3 metabolites [337]. Hence, there may be other metabolites such as LCA that bind vDBP and reduce the efficiency of vDBP to perform its biological tasks in clearing Aβ plaques. Interestingly, perhaps as a compensatory mechanism, serum and CSF levels of vDBP increase in AD [338]. More precisely, the levels of vDBP increase from 0.6 ± 0.0 μg/ml in control patients to 1.2 ± 0.1 μg/ml in patients with AD [339]. However, this may not be high enough, then drug delivery systems such as PLGA nanoparticles may be employed to deliver vDBP to the brain to reduce accumulation of Aβ in the brain [340]. Therefore, if the functionality of vDBP is restored by reducing serum levels of LCA, then therapeutic benefits such as improvements may be observed in cognitive functions of patients with AD. It is important to start early treatment, as at the late stages, the brain is too compromised to take benefits from the drug [341].

THERAPEUTIC APPROACHES

Administration of TUDCA at the dose of 750 mg/day is safe in patients with liver cirrhosis [342]. This did not change serum levels of lithocholic acid, but decreased serum levels of CDCA and cholic acid at doses of 500 mg/day or higher [343]. Furthermore, TUDCA improved glucose homeostasis in streptozotocin-induced mouse AD model [344]. Certain bile acids have anti-inflammatory and antioxidative activities such as TUDCA and TCA [345]. However, it should be noted that administration of

Table 1
List of drugs that can reduce serum bile acids including LCA

Drug	Dose/day	Mechanism of action/	Duration/ Days-weeks	Remarks	Limitations	Ref
Cholestyramine	16 g	Bile acid binding resin	3 weeks	Decreases serum levels of lithocholic acid. The change is not large	Unpleasant taste, diarrhea, bloating, Vitamin K deficiency	[347, 361]
UDCA	900 mg	Stimulation of biliary secretion of bile Acids	15 days	Reduced serum LCA from 20 μmol/L to 3 μmol/L (normal level is 1 μmol/L)	Not observed, rare mild diarrhea	[362–365]
Colestipol	7.5 g	Bile acid sequestrant	6 days	Reduced serum bile acids	Unpleasant taste	[366, 367]
Vancomycin	1.5 g	Non-absorbable antibiotic affecting intestinal microbiota	7 days	Serum secondary bile acids decreased	Diarrhea	[368]
Atrovastatin	40 mg	Activation of pregnane X nuclear receptors	12 weeks	Reduce serum GLCA from 42 ng/ml to 34 ng/ml	Increased serum alanine aminotransferase levels (liver damage)	[369, 370]
Dexamethasone	12 mg	By reducing serum estriol and estradiol levels and their effects on the liver	10 days (oral)	Reduced serum bile acids	Suppression of pituitary-adrenal axis	[371, 372]
S-adenosyl-L-methionine	800 mg	By blocking the effects of estrogen on the liver	10 days (i.v.)	Reduced total serum bile acids	No side effects	[373]
Elobixibat	10 mg	SLC10A2 inhibitor	14 days	Potential reduction of serum LCA due to shorter secondary bile acid time in the intestine	No serious adverse events, some or mild GI discomforts	[355, 357, 374, 375]
Activated charcoal	150 g	Preventing bile acid enterohepatic recirculation	8 days	Reduced serum bile acids	Preventing absorption of key nutrients such as vitamin K, and alarming black stool!	[376, 377]
Obeticholic acid	5-10 mg	Selective farnesoid X receptor agonist	12 months	Significantly reduced plasma bile acid levels including C4	Pruritus	[346, 378]
Maralixibat	20 mg	Apical sodium dependent bile acid competitive inhibitor	13 weeks	Reduced serum bile acids from 52 ng/ml to 33 ng/ml	Gastrointestinal disorder (abdominal pain)	[379]
GSK2330672	Starting with 90 mg then 180 mg	Ileal bile acid transporter inhibitor	3 days at 90 mg then increase to 180 mg until day 14	Reduced serum bile levels by 50% (from 30 μM to 15 μM)	Diarrhea	[380]

Colesevelam	3.75 g	Anion-exchange resin with strong bile acid-binding capacity	3 weeks	Reduced total serum bile acids from 155 μmol/L to 140 μmol/L.	Mild stool change	[381]
Rifampicin	600 mg	Hydroxylation of hydrophobic bile acids and reducing their cytotoxicity	1-2 weeks	Reduced total and conjugated bile acids	No adverse effects	[382, 383]
Guar gum	15 g	Binds with bile acids in the intestinal lumen	At least 10 days	Prevented increase of serum bile acids levels	Mild abdominal distress	[384]
Cilofexor	100 mg	Non-bile acid FXR agonist	12 weeks	Reduced primary and secondary serum bile acids by 50% and 40%, respectively	Advert events (mainly pruritus) were similar to placebo	[385, 386]
NGM282	3 mg injection	FGF-19 mimetics	12 weeks	Significantly reduced total serum bile acids (conjugated bile acids and DCA significantly reduced, LCA was also reduced but not significantly)	Gastrointestinal symptoms such as diarrhea and injection site reactions	[387]
Bezafibrate	400 mg/day plus UDCA (600 mg/day)	PPAR agonist	3 months	Reduced serum bile acids including LCA (did not reach statistical significance)	Adverse events were not reported when bezafibrate used with UCA, however, fibrates on their own may cause hepatotoxicity, increased risk of developing gallbladder disease and venothromboembolic disease (pulmonary embolism)	[388–390]
MBX-8025	200 mg	PPARσ agonist	12 weeks	Reduced serum C4 and bile acid levels	Frequent adverse events were pruritus, diarrhea, and nausea	[391]
A4250	3 mg	Apical sodium dependent bile acid transporter inhibitor	1 week	Reduced serum bile acids	Abdominal symptoms including increased bowel movements	[392]
Phenobarbitone	3 mg/kg	Hepatic smooth endoplasmic reticulum enhancement, an increase in hepatic cytochrome P-450 content	2 weeks	Reduced serum bile acids (did not reach statistical significance)	Not reported	[393]

bile acids such as obeticholic acid (Supplementary Figure 3) comes with pruritus, which is common adverse event with FXR agonists [346]. Administering cholestyramine 16 g/day for three weeks decreased serum levels of lithocholic acid, but generally the change was not significant [347]. However, the limitations of the therapy are: unpleasant taste, diarrhea, and bloating. As 7α-hydroxylation by CYP7A is necessary for the conversion of cholesterol to bile acid, cholestyramine is an inducer of CYP7A, which is also active against 27-hydroxycholesterol. This is in addition to CYP7B [348]. Therefore, administration of cholestyramine will lead to more acidic pathway, and hence more CDCA, and potentially more LCA, and this could be an explanation why administration of cholestyramine does not significantly reduce serum LCA levels [81]. On the other hand, the probiotics cocktail VLS#3 could promote excretion of bile acids [349] and reduce LCA levels in AD patients (by increased bowel movement).

Bile acid therapy will be useful when serum C24 bile acid levels are low. Bile acid therapy will help absorption of lipids and vitamins [20]. Oral administration of non-absorbable antibiotics (vancomycin and polymyxin B) reduced amounts of LCA and DCA in the feces of mice after 5 days. This was due to the dysbiosis induced by the antibiotics. It should be noted that reduced levels of LCA and DCA changed the expression of hepatic enzymes such as CYP2b10, CYP3a25, and CYP51a1, which could affect metabolism of other drugs taken by the patient [350]. Can treatment with CDCA increase serum levels of LCA? There is a possibility, as treatment with DCA increases the amounts of DCA in the bile [351]. Therefore, treatment with CDCA may increase the content of this bile acids in the bile and consequently amounts of LCA in the blood. Sadeghi et al. (2020) also found that bile acid therapy by CA or sodium deoxycholate reduced acetylcholinesterase activity in the hippocampus of AD rat model, and this therapy also reduced the Aβ load in the brain of the animal models [352].

Table 1 presents a list of drugs that have been administered in different diseases such as intrahepatic cholestasis pregnancy and chronic idiopathic constipation to reduce serum levels of bile acids or where reduction of serum bile acids occurred unintentionally. Patients with AD may suffer from constipation, and this could be due to dysregulation of mAChr signaling pathways and ER stress response [353]. Therefore, treatment with drugs such as elobixibat (an inhibitor of ileal bile acid transporter [354]) may reduce the serum levels of LCA due to increased bowel movement, as it is suggested that constipation increases LCA serum levels due to greater LCA reabsorption [355]. High bile acid levels in feces are associated with diarrhea [356]. Importantly, delayed colon transit elevates serum C4 levels, indicating altered/increased bile acid synthesis and further elevation of serum LCA levels [357]. Interestingly, serum levels of LCA decreased below the detection level by applying transcutaneous neuromodulation [355]. In this method, electrical stimulation was delivered noninvasively via surface electrodes placed at both the acupoint ST36 and the posterior tibial nerve using an external watch-size stimulator. As an alternative method, total plasma exchange reduced serum bile acids by replacing the patient's plasma volume with 5% albumin fluid [358]. This is known as therapeutic plasma exchange which is a procedure that the patient's blood is passed through an apheresis machine. The filtered plasma is removed and discarded with reinfusion of red blood cells along with replacement fluid such as plasma or albumin in to the patient [359].

Elafibranor (GFT505) is agonist of PPARα /σ [360], and has been investigated in patients with primary biliary cholangitis in a phase II clinical trial (NCT03124108). Change in serum bile acids was a secondary outcome measure, but the report is due for publication. Elafibranor may reduce serum bile acid levels, as GENFIT reported reduction of serum C4 levels in the patients at the dose of 80 or 120 mg/day for 12 weeks [360].

CONCLUSIONS

Recent clinical studies have shown that serum bile acid levels increase in patients with AD including toxic bile acids such as LCA, perhaps due to the peroxisomal dysfunction. Hydrophobic bile acids such as DCA alter the permeability of the BBB to molecules that normally do not cross the BBB. Hydrophobic bile acids activate FXR signaling in the brain, which leads to recued brain levels of hydrophilic bile acids. Hydrophobic bile acids block NMDARs, with LCA being the most potent. NMDARs are crucial for the formation of temporal memory. Molecular modelling suggests that LCA has the ability to form a complex with E2 receptors, but without physiological actions, preventing estrogen from exerting its role in cognitive function and memory. LCA may reduce the expression of ABCA1,

promote the formation of Aβ fibrils, leading to accumulation of Aβ plaques in the brain at the early stages of AD. The plasma or serum levels of 24-OHC were significantly lower in patients with AD and elevated serum bile acids levels have the ability to reduce synthesis of 24-OHC via activation of FXR. Bile acids have the ability of activating TGR5 and leading to neuroprotection and diminishing of inflammation. However, the expression of TGR5 decreases in the brains of patients with AD, with potential contributions from toxic bile acids. Therefore, the benefits from elevated serum bile acids may not be observed. Toxic bile acids may inhibit the activation of the SHH, by preventing the interaction of potent endogenous oxysterols with SMO protein. There are several approaches to reduce serum bile acids such as the use of transcutaneous neuromodulation, administration of cholestyramine (16 g/day), or obeticholic acid (5-10 mg/day).

FUTURE DIRECTIONS

Further studies should be conducted aiming to reduce serum levels of bile acids in patients with AD. These studies should include evaluation of the clinical outcomes, along the line of that undertaken by Marksteiner et al. (2018) [39], i.e., measuring the serum bile acids of patients with AD and recording basic cognitive scores (e.g., MMSE) at the beginning of trial. The intervention would be administering a therapeutic agent to reduce serum bile acids for a period of six months, followed by cognitive assessment scores, in parallel with serial measurements of serum bile acids. An alternative approach could be monitoring serum bile acid levels of individuals regularly, and establish association between serum bile acid levels and cognitive function, given link between secondary bile acids in the gut and brain [394]. Another direction would be to promote exercise in elderly subjects and monitoring serum bile acids and cognitive function. It has been shown that recreational physical activity is inversely associated with total fecal bile acid concentrations [395], and exercise is also linked to the reduction of total serum bile acid levels [396]. Optimizing the diet also could be another safe and inexpensive means of further investigation in patients with AD, as diet affects serum bile acid levels (vegans having higher serum bile acids compared to omnivores) [397].

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SUPPLEMENTARY MATERIAL

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