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CRITICAL REVIEW

A review of the putative antiseizure and antiepileptogenic mechanisms of action for soticlestat

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Abstract

Soticlestat (TAK-935) is a potent and selective inhibitor of cholesterol 24-hydroxylase (CYP46A1), an enzyme primarily expressed in the brain that catabolizes cholesterol to 24*S*-hydroxycholesterol (24HC). In the ELEKTRA phase II clinical trial, soticlestat reduced seizure frequency in patients with developmental and epileptic encephalopathies, and two phase III studies evaluating the safety and efficacy of soticlestat in Dravet syndrome (SKYLINE) and Lennox–Gastaut syndrome (SKYWAY) have recently been completed. The exact mechanism of action by which soticlestat exerts pharmacological benefits remains undetermined. In this review, we assess the available preclinical evidence and present a working hypothesis for the antiseizure effects of soticlestat. The data support three potential mechanisms of action: (1) normalization of the seizure threshold via reduction of 24HC levels in the brain; as 24HC acts as a potent and selective positive allosteric modulator of glutamate N-methyl-D-aspartate receptors, reduction of 24HC levels by soticlestat may lead to decreased hyperexcitability and elevated seizure thresholds; (2) restoration of glutamate sequestration from the synaptic cleft; accumulation of glutamate in the synaptic cleft enhances neural excitation and can contribute to neurotoxicity; soticlestat may inhibit conversion of cholesterol to 24HC in the membrane lipid raft microdomain and help to preserve it, consequently reducing excessive glutamate excitation; and (3) suppression of neuroinflammation via reduction of inflammatory cytokine release. These potential mechanisms of action warrant further investigation.

KEYWORDS

24*S*-hydroxycholesterol, cholesterol 24-hydroxylase, epilepsy, glutamate, seizures, soticlestat

1 | INTRODUCTION

Soticlestat (TAK-935) is a first-in-class potent and selective inhibitor of cholesterol 24-hydroxylase (CH24H; CYP46A1), an enzyme primarily expressed in the brain that catabolizes cholesterol to 24*S*-hydroxycholesterol (24HC). Two phase III studies evaluating the safety and efficacy of soticlestat in Dravet syndrome (SKYLINE,

NCT04940624)[1] and Lennox–Gastaut syndrome (SKYWAY, NCT04938427)[2] have recently been completed. The exact mechanisms of action by which soticlestat exerts pharmacological benefits, such as reducing seizure frequency as seen in the ELEKTRA phase II study in patients with Dravet syndrome and Lennox–Gastaut syndrome,[3] remains to be fully elucidated. Here, we review the available evidence and present a working hypothesis for the mechanism of action of soticlestat.

The enzymatic reaction of CH24H, the target for soticlestat, exerts at least two biologically meaningful consequences: reduction of the bioactive metabolite 24HC and maintenance of local cholesterol content in cell membranes within the brain. There is increasing evidence to suggest that 24HC is a signal mediator for N-methyl-D-aspartate (NMDA), retinoic acid receptor-related orphan receptors (RORs), and liver X receptors (LXRs), which is discussed in Section 5. In addition, brain cholesterol is tightly controlled to remain constant. For example, when CH24H was deleted in mice, total brain cholesterol remained unchanged.[4] It was determined this occurred via reduction in de novo cholesterol synthesis, indicating the critically important role of cholesterol homeostasis.[4] Furthermore, a wide range of membrane proteins, including caveolins, have been associated with functioning of the membrane lipid raft, a cholesterol-rich microdomain.[5] Consequently, catabolism of membrane cholesterol by CH24H impacts various biological processes, including local regulation of the glutamate NMDA receptor (NMDAR), which is discussed in Section 7. Herein, we describe CH24H as the target of soticlestat, summarize soticlestat's pharmacological effects in preclinical studies, and discuss a plausible mechanism of action for soticlestat.

2 | SEARCH CRITERIA AND METHODOLOGICAL DESCRIPTION

To search for relevant literature, the PubMed database was used for exhaustive coverage. The literature search was conducted with combinations of keywords including "CH24H", "cholesterol 24-hydroxylase", "CYP46A1", "cholesterol 24-monooxygenase", "24*S*-hydroxycholesterol", "cholesterol", "glutamate", "seizures", "convulsion", "epilepsy", "inflammation", and "neuroinflammation". Selection was made first by reading the abstracts of the searched literature and second by reading sections of each publication for relevance. References in publications on important topics were sometimes cited as secondary sources.

Key points

- Soticlestat, a selective inhibitor of cholesterol 24-hydroxylase, reduces catabolism of cholesterol to 24HC.
- Clinical and preclinical data support three potential antiseizure mechanisms of action of soticlestat: (1) reduction of 24HC, a direct modulator of signal transduction via glutamate NMDA receptors; (2) restoration of glutamate sequestration from the synaptic cleft and preservation of the membrane lipid raft microdomain; and (3) suppression of neuroinflammation via reduction of 24HC levels, normalization of inflammatory cytokine release, and glutamate regulation.

3 | HOMEOSTASIS OF CHOLESTEROL IN THE BRAIN AND CH24H

The brain is particularly rich in cholesterol; approximately 25% of the body's total cholesterol resides in the brain.[6] Most brain cholesterol (approximately 70%–80%) is associated with myelin sheaths.[7] The central nervous system is isolated from the circulatory system by the blood–brain barrier, thereby preventing the movement of cholesterol between blood and brain.[8] Consequently, >95% of brain cholesterol is synthesized de novo within astrocytes.[8] Cholesterol synthesis begins from conversion of acetate through 3-hydroxy-3-methylglutaryl coenzyme A, mevalonate, lanosterol, and divergence to either the Bloch or the Kandutsch–Russel pathway through desmosterol or 7-dehydroxycholesterol, respectively (Figure 1).[9] Cholesterol is a constituent of the cell membrane and controls membrane fluidity, thickness, and flexibility,[10] all of which are important determinants for cellular functions. Cholesterol, together with sphingolipids, organizes a microdomain of the cell membrane called the lipid raft, which serves as a functional domain for signal transduction[11] and vesicular transport.[12]

Similarly, cholesterol in the brain needs to be converted into oxysterols (polar molecules that can traverse membrane barriers) for excretion from the brain.[13,14] Among the brain oxysterols formed,[15] 24HC is the major metabolite in the brain. Furthermore, it is estimated that approximately 80% of cholesterol excretion from the human brain is mediated through hydroxylation by CH24H.[4] In support of this concept, CH24H knockout mice demonstrated a reduction in cholesterol efflux from the brain by 64%.[16]

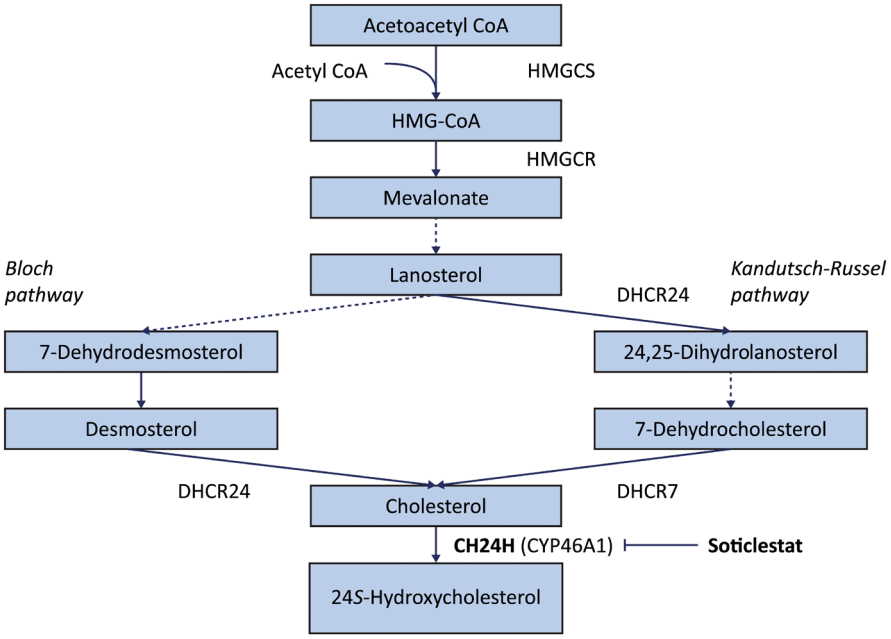


FIGURE 1 The cholesterol pathway in the brain. CH24H, cholesterol 24-hydroxylase; CoA, coenzyme A; DHCR, dehydrocholesterol reductase; HMG, 3-hydroxy-3-methylglutaryl; HMGCR, HMG-CoA reductase; HMGCS, HMG-CoA synthase.

4 | CH24H (CYP46A1) EXPRESSION AND ITS REGULATION

CH24H, also known as CYP46A1 (EC 1.14.13.98), is a monooxygenase that hydroxylates the sidechain of cholesterol to form 24HC. Expression of CH24H was shown to localize predominantly in the brain, with residual RNA expression detected in some peripheral organs.[17,18] In the brain, CH24H expression is high in pyramidal neurons of the hippocampus and cortex, in Purkinje cells of the cerebellum, and in hippocampal and cerebellar interneurons.[19] The brain-selective expression pattern of CH24H corroborates the experimental results finding that conversion of cholesterol to 24HC by CH24H is the dominant mechanism for excretion to the periphery.[4] CH24H expression is also under developmental regulation. CH24H protein levels are low at birth and gradually increase to steady-state levels toward early childhood in both humans and mice. Brain 24HC levels increase concurrently with CH24H protein expression, whereas serum 24HC levels show a steep peak at approximately 2 weeks of age in mice.[17] The lack of correlation between CH24H expression and serum levels of 24HC implies additional regulatory mechanisms for CH24H-mediated cholesterol catabolism. CH24H was shown to translocate to the plasma membrane upon intracellular Ca^{2+} mobilization evoked by glutamatergic neurotransmission in primary hippocampal cultures. CH24H caused a small, but significant, loss of cholesterol and release of 24HC to the extracellular milieu.[20] Furthermore, knockdown of CH24H in rat hippocampal neurons prevented these changes.[20] Additionally, chronic glutamate exposure was found to induce CH24H expression through cAMP response element-binding protein activation.[21] Collectively, these results suggest the possibility that CH24H influences neural activity-dependent regulation of cellular activities.

Alteration of CH24H expression has also been reported in various neurological diseases. In Alzheimer disease, CH24H expression was found to be decreased in neurons and increased in astrocytes.[22,23] Reduced expression of CH24H in patients with Huntington disease was reported in the striatum and cerebral cortex, a brain region in which significant neurodegeneration occurs.[24,25] However, in postmortem brains, it was not conclusive whether reduced CH24H expression caused neuronal cell death or reflected the loss of neuronal cells within the lesion. There is evidence that CH24H is upregulated in surviving neurons in surgically resected hippocampi from patients with temporal lobe epilepsy and hippocampal sclerosis, as well as in the hippocampus of the intra-amygdala kainate (IAK) mouse model of acquired epilepsy.[26] These findings do not support that the enzyme is responsible for neuronal cell loss in epilepsy. Nevertheless, gene therapy delivery of CH24H into the striatum of the zQ175 Huntington disease mouse model revealed normalization of dysregulated gene expressions, including *FAS* and *LDLR LXR*-target genes, proteasome genes *UB2L6* and *PSMB9*, the autophagy gene *TRIM21*, and *STAT3*, involved in the immune response.[27] CH24H gene therapy also increased synapse density, improved vesicle trafficking, and cleared mutant Huntington aggregates,[27] suggesting there are beneficial effects to increasing expression of CH24H. In other rodent disease models, increased expression of CH24H was observed in demyelination plaques in the spinal cord of the experimental autoimmune encephalomyelitis model,[28] in the cerebral cortex of the hypoxia–ischemia model,[29]

and in traumatic brain injury lesion sites in a rat model.[30] Interestingly, reduced expression of CH24H was observed in the cerebral cortex of patients with spinocerebellar ataxia.[31] 24HC levels in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis (ALS) were lower than in a healthy control group,[32] suggesting reduced CH24H activity in ALS. However, CH24H transcript levels in patients with ALS were not different from those of a healthy control group.[33] Further studies are required to elucidate the roles of CH24H expression in the etiology and progression of diseases of the central nervous system.

5 | FUNCTIONS OF 24HC

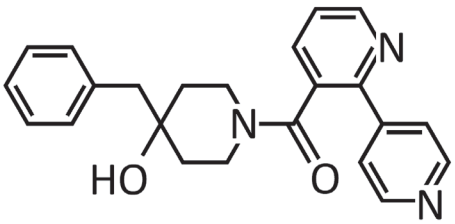
Cholesterol oxidation is the major pathway for brain excretion and eventual conversion into bile acid. This process is important for maintaining the homeostasis of brain cholesterol. Conversely, hydroxylated cholesterols and other intermediates can serve as signaling molecules.[14,15,34] In the brain, 24HC,[35] 25HC,[36] and 27HC[37] are hydroxycholesterols formed from enzymatic conversion of cholesterol. Among those, 24HC is the most abundant and implicated in a variety of biological processes.

It was first reported that 24HC binds to the LXRs α and β and can activate each receptor with half-maximal effective concentrations of 4 μmol·L^{-1} and 3 μmol·L^{-1}, respectively.[38] Subsequently, it was reported that 24HC also binds to the RORs and acts as an inverse agonist on these constitutively active nuclear receptors.[39] As both LXRs[40] and RORs[41] are implicated in the cholesterol metabolic pathway, the role of oxysterols in cholesterol feedback control through LXRs has been investigated.[42] A variety of oxysterols bind and function on LXRs, making it difficult to delineate the effects of a single oxysterol. For example, CH24H transgenic mice were found to have four- to sevenfold higher 24HC levels. However, no notable changes in *LXR* gene expression were detected in the brain or liver.[43] At the cellular level, high concentrations of 24HC (≥10 μmol·L^{-1}) were reported to induce necroptosis (programmed cell death) in SH-SY5Y neuroblastoma cells[44] and caspase-8-dependent apoptosis in Jurkat T cells.[45] Additionally, it was reported in 158 N murine oligodendrocytes that 24HC induced oxiapoptophagy, a type of cell death induced by oxysterols that involves simultaneous oxidative stress, apoptosis, and autophagy.[46] These cell deaths were protected by α-tocophenol,[46–50] implying a role of lipid peroxidation in the induction of cell death by 24HC. In a primary neuronal culture assay mimicking ischemic conditions by oxygen and glucose deprivation, addition of 2 μmol·L^{-1} 24HC exacerbated NMDAR-dependent excitotoxicity. Similarly, increased 24HC synthesis lowered cell survival rate, whereas reduced 24HC synthesis attenuated cell death.[51] On the contrary, low concentrations (10 nmol·L^{-1}–1 μmol·L^{-1}) of 24HC had protective effects on cell death triggered by staurosporine[52] and 7-ketocholesterol[53] in SH-SY5Y cells. As discussed below in Section 7, 24HC was found to serve as a direct modulator of neuronal and inflammatory signal transduction and exhibited the ability to function as a positive allosteric modulator (PAM) on NMDARs[54] and αVβ3 integrin receptors.[55] These modulatory roles of 24HC, and their potential significance in epilepsy, are discussed in Section 7.

6 | SOTICLESTAT, A SELECTIVE INHIBITOR OF CH24H

The potent CH24H inhibitor soticlestat (4-benzyl-4-hydroxypiperidin-1-yl)(2,4′-bipyridin-3-yl)methanone was discovered using a structure-based drug design approach. Soticlestat inhibits CH24H with a half-maximal inhibitory concentration value of 7.4 nmol·L^{-1} and is orally bioavailable and brain-penetrant with a CLogP-value of 1.4.[56] Soticlestat selectively inhibits CH24H (CYP46A1) over other cytochrome P450 enzymes (Table 1). In addition,

TABLE 1 Selective inhibition of soticlestat.



Soticlestat

CYP	IC_{50}, nmol·L^{-1}	CYP	IC_{50}, nmol·L^{-1}
2C8	62 000	11B1	>10 000
2C9	19 000	11B2	>10 000
2D6	>100 000	17	>10 000
3A4	66 000	21	>10 000
1A2	>100 000	19	ND[a]
2C19	14 000	46A1	7.4

Abbreviations: CYP, cytochrome P450; IC_{50}, half-maximal inhibitory concentration; ND, not determined.

[a]Sixty-eight percent inhibition at 10 μmol·L^{-1}.

soticlestat displays minimal inhibition of human liver microsomes.[57]

The APP/PS1 double transgenic mice, originally developed as an Alzheimer disease model, are also known for seizure-related sudden death[58] and an excitatory/inhibitory imbalance.[59] Chronic administration of soticlestat at 10 mg/kg once daily markedly improved the survival rate of APP/PS1 double transgenic mice and reduced 24HC brain concentrations by approximately half.[60] The observation was consistent with the finding that triple mutant CH24H$^{-/-}$ APP/PS1 mice had significantly extended lifespans compared with APP/PS1 double mutants.[61] These results prompted investigation into soticlestat's effect on seizure generation in APP/PS1 mice.

Potassium chloride (KCl) brain perfusion evoked a ≥20-fold increase in extracellular glutamate in the brains of APP/PS1 mice but not in wild-type mice. Pretreatment with soticlestat suppressed the glutamate surge in APP/PS1 mice brain after KCl perfusion.[60] It was hypothesized that regulation of interstitial glutamate was impaired in APP/PS1 mice and soticlestat normalized the impairment. Notably, in the presence of an astrocytic glutamate uptake inhibitor, DL-threo-β-benzyloxyaspartate (TBOA), the effect of soticlestat was abolished.[60] TBOA is a blocker of excitatory amino acid transporters (EAATs), and therefore it was hypothesized that soticlestat may affect functional regulation of EAATs in astrocytes under certain pathological conditions.

The reduced seizure frequency and concomitant protection from seizure-related death in APP/PS1 mice led us to investigate soticlestat's effects in the Dravet mouse model, which exhibits frequent spontaneous seizures and a sudden unexpected death in epilepsy-like phenotype. Pathogenic variants in the sodium channel gene *SCN1A* can result in Dravet syndrome, a developmental and epileptic encephalopathy.[62] Heterozygous *Scn1a*$^{+/-}$ mice recapitulate several phenotypes of Dravet syndrome, including spontaneous seizures, premature lethality, and hyperthermia-induced seizures.[63] Soticlestat treatment reduced seizure burden to almost zero, completely prevented premature lethality, and increased the temperature threshold for heat-induced seizures in two distinct *Scn1a* Dravet mouse models.[64] Improvement in several phenotypic outcomes with soticlestat treatment in various syndrome-specific mouse models, including APP/PS1 and two separate Dravet models, implies a possibility that soticlestat has multimodal actions that can normalize neural hyperexcitation under certain pathological conditions.

Soticlestat was also evaluated in a variety of standard epilepsy screening paradigms (Table 2).[65] Unexpectedly, soticlestat was only beneficial in the Frings audiogenic model[66] and in the pentylenetetrazol (PTZ) kindling model.[67] Soticlestat was ineffective in the maximal electroshock model of generalized tonic–clonic seizures, a "gold standard" in epilepsy drug screening,[65,68] the subcutaneous PTZ model of myoclonic seizures,[69] and the 6-Hz psychomotor seizure assays.[70] However, it should be noted that in the maximal electroshock assay, 75% (6/8) of vehicle-treated mice died, whereas no deaths were observed in the soticlestat arm,[65] inferring a protective effect of soticlestat from seizure-related sudden death. In a

TABLE 2 Effect of soticlestat in rodent models of seizures.[65]

Test	Species	Soticlestat	Protected/tested, *n*
Maximum electroshock seizures model	Mouse[a]	Vehicle	0/8
		30 mg/kg/day	0/8
	Rat	Vehicle	1/8
		100 mg/kg/day	0/8
Subcutaneous pentylenetetrazol model	Mouse	Vehicle	0/8
		30 mg/kg/day	0/8
6-Hz model of psychomotor seizures (32-mA stimulation intensity)	Mouse	Vehicle	0/8
		30 mg/kg/day	0/8
6-Hz model of psychomotor seizures (44-mA stimulation intensity)	Mouse	Vehicle	0/8
		30 mg/kg/day	0/8
Amygdala kindling	Rat	Delayed kindling acquisition but no effect after discharge duration (100 mg/kg orally once daily)	
Intra-amygdala kainate injection model	Mouse	Delay in epilepsy onset, threefold reduction of seizures during treatment, and fourfold reduction of spontaneous seizures several weeks after drug washout Reduction of established chronic seizures	
Frings audiogenic seizure model	Mouse	Seizure protection with ED_{50} = 10.7 mg/kg (3-day pretreatment)	
Theiler murine encephalomyelitis virus model	Mouse	Significant protection in seizure severity and latency to onset (30 mg/kg from day 0 to 7 postinfection)	

Note: Modified from Nishi T, Metcalf CS, Fujimoto S, Hasegawa S, Miyamoto M, Sunahara E, et al. Anticonvulsive properties of soticlestat, a novel cholesterol 24-hydroxylase inhibitor. *Epilepsia* 2022;63(6):1580–1590,[65] https://onlinelibrary.wiley.com/doi/full/10.1111/epi.17232, © 2022 Takeda Pharmaceutical Company Limited, and made available under the terms of the Creative Commons Attribution-NonCommercial license.

Abbreviation: ED_{50}, median effective dose.

[a]No deaths occurred in the soticlestat treatment arm, whereas six of eight mice died in the vehicle-treated group.

separate study, soticlestat was tested in the Theiler murine encephalomyelitis virus infection model,[71] an infection-induced epilepsy model with both acute handling-evoked seizures and eventual epileptogenesis with spontaneous, recurrent seizures.[72] Soticlestat reduced acute seizure burden and delayed acute seizure manifestation. The study also reported protective effects of soticlestat on chronic behavioral deficits after 36 days of drug-free period, but no results on its effects on spontaneous seizures in this model were described. In line with these results, soticlestat demonstrated both antiseizure and disease modification effects in IAK-injected mice after a transient post-status epilepticus administration period of soticlestat.[26] In this golden standard model of acquired epilepsy, soticlestat exerted delay in epilepsy onset, a threefold reduction in the number of seizures during treatment (antiseizure effect), fourfold reduction in the total number of spontaneous seizures several weeks after drug washout (disease modification effect), and reduction of established chronic seizures. These effects were associated with shortening of seizure duration and neuroprotection. The abovementioned results illustrate that soticlestat has characteristics distinct from conventional anticonvulsants in that it exerts antiseizure effects in models wherein either a genetic or other insult leads to chronic network hyperexcitability and lowered seizure threshold. As such, it may also be useful for the treatment of diseases associated with seizures and epilepsy-associated comorbidities.

7 | SOTICLESTAT POSSIBLE MECHANISMS OF ACTION

7.1 | NMDAR signal modulation

There was a strong correlation observed in the PTZ kindling experiment between brain levels of soticlestat and brain levels of 24HC. In the same experiment, brain 24HC levels were correlated with cumulative seizure scores.[65] Therefore, it is likely that the antiseizure effect of soticlestat is primarily exerted through reduction of brain 24HC. In 2013, 24HC was reported to be a potent, direct, and selective PAM of NMDARs, the predominant glutamate-gated ion channels in the brain that contribute to excitatory neural transmission.[54] Exogenously added 24HC potentiated hippocampal excitatory postsynaptic currents in vitro in a concentration-dependent manner between 10 $nmol \cdot L^{-1}$ and 10 000 $nmol \cdot L^{-1}$, equivalent to physiological concentrations estimated in the adult brain.[73] Electrophysiological examination revealed that 24HC required >20 s to reach maximal effect, and potentiation was poorly reversible.[54] Therefore, it is reasonable to speculate that once 24HC interacts with NMDARs, the positive regulatory effect persists and contributes to longer term elevation of excitatory tone of synapses. It is widely accepted that glutamate-mediated neuronal hyperexcitation, through ionotropic NMDARs, results in lowering the seizure threshold and evoking seizures. Agonists for NMDARs elicit, and antagonists reduce, seizures in both animals and humans.[74] It is postulated that activation of NMDARs by 24HC increases hyperexcitability and lowers seizure threshold. It is further hypothesized that soticlestat, by reducing the production of 24HC through inhibiting CH24H, elevates seizure threshold. Reduction of NMDA-mediated excitability may also contribute to the observed antiepileptogenic effects that have been observed in the amygdala and PTZ kindling studies (Figure 2).

Functions of endogenous 24HC were subsequently investigated using hippocampal slices prepared from wild-type and CH24H knockout mice.[73] As expected, 24HC levels were greatly reduced in the hippocampal slices of the CH24H knockout (2.0 ± .5 ng/mg protein) compared with wild-type (326.0 ± 16.2 ng/mg protein) mice. Slices from CH24H knockout mice showed a significantly smaller NMDAR to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) excitatory postsynaptic current ratio than slices from wild-type mice, indicating that reduced endogenous 24HC in knockout mice lowered NMDAR function and hyperexcitability. However, notable changes in neuronal intrinsic excitability, spontaneous neurotransmission, or long-term potentiation were not seen. These observations seem to agree with the mild phenotype of CH24H knockout mice, which exhibited mildly impaired learning and hippocampal long-term potentiation.[4] These milder phenotypes, even under nearly complete reduction of 24HC in the brain, provide a rationale for the safe use of soticlestat. Its safety is also supported by the subtle change of transcriptome in the mice treated with soticlestat.[26] At the same time, these observations may raise a question as to whether reducing PAM effects at NMDARs is meaningful in disease control. It should be noted, however, that CH24H is developmentally regulated and there is a steep peak of serum 24HC at approximately 2 weeks postnatally, implying roles for CH24H/24HC in the developing mouse brain.[17] Thus, the brain slices used in the experiments might have developed differently from those of a normal brain and adapted to the CH24H-free conditions. It is also noteworthy that Sun et al. reported that the NMDAR PAM site in ex vivo slices was not saturated by 24HC despite high concentrations.[73] It seems that 24HC engagement of the PAM site is not just simply regulated by the availability of 24HC. In this context, it is interesting to note that cellular stress from excitatory neurotransmission reportedly contributes to cholesterol loss in hippocampal neurons by mobilizing

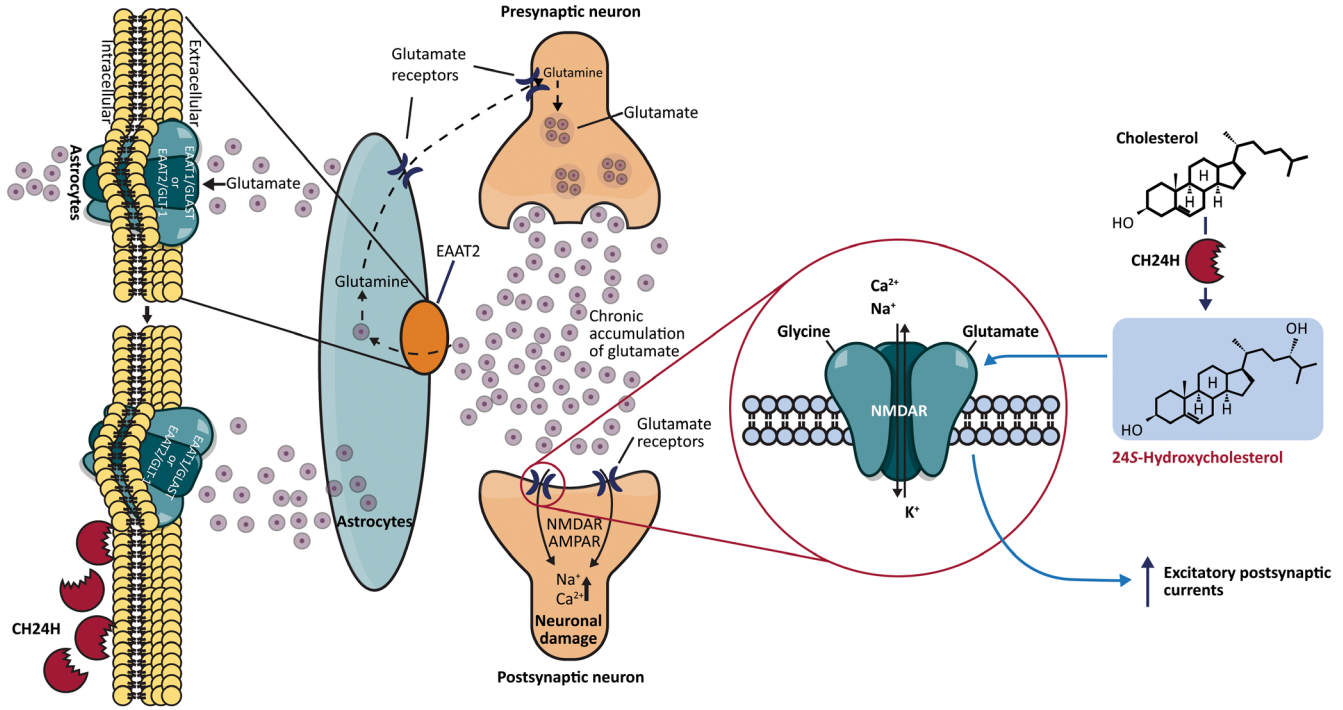


FIGURE 2 N-methyl-D-aspartate receptor (NMDAR) signaling, glutamate-enhanced neural excitation and lipid raft integrity. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CH24H, cholesterol 24-hydroxylase (CYP46A1); EAAT, excitatory amino acid transporter; GLAST, glutamate–aspartate transporter; GLT-1, glutamate transporter 1.[75] Part of this figure is adapted from Vallée A, Vallée J, Guillevin R, Lecarpentier Y. Riluzole: A therapeutic strategy in Alzheimer's disease by targeting the WNT/β-catenin pathway. *Aging* (*Albany NY*) 2020;12:3095–3113, https://doi.org/10.18632/aging.102830, © 2020 Vallée et al., and made available under the terms of the Creative Commons Attribution License (CC BY 3.0).[75]

CH24H to the plasma membrane.[76] Thus, it seems to be a vicious cycle in which exaggerated excitatory neurotransmission causes cellular stress that mobilizes CH24H to the plasma membrane, then CH24H locally converts cholesterol to 24HC, further escalating glutamate-mediated excitatory transmission. This dysregulated glutamatergic hyperactivation is often seen in pathogenic conditions. Beneficial effects of soticlestat are seen in preclinical models with persistent perturbations such as genetic, repetitive kindling, status epilepticus, and virus infection followed by neuroinflammation. More studies are warranted to delineate where and how 24HC is generated from membrane cholesterol and supplied to NMDARs in situ under both physiological and pathogenic conditions.

7.2 | Lipid raft integrity and glutamate sequestration

Dysregulation of ambient glutamate in the synaptic cleft is ascribed to hyperexcitation and seizures.[77] Once released from the presynaptic terminal, glutamate is immediately cleared from the intersynaptic space by transporters on astrocytes. Hence, astrocyte end feet localize in close proximity to pre- and postsynaptic membranes, which is called the tripartite synapse.[78] Glutamate is recycled between astrocytes and neurons through a process known as the glutamate–glutamine cycle, and the reuptake process is critical for homeostasis of neurotransmission.[79] EAAT2 (EAAT2/solute carrier family 1 member 2/glutamate transporter 1) is reportedly responsible for the clearance of >90% of glutamate,[80] and its association with the cholesterol-rich lipid membrane raft microdomain is vital for its functioning.[81] Of note, the predominant cholesterol-catabolizing enzyme in the brain, CH24H, is induced in astrocytes under certain conditions, such as in reactive astrocytes in the aged brain,[82] Alzheimer disease,[23] the injured brain,[30] and temporal lobe epilepsy.[26] Interestingly, it was reported that increased expression of CH24H in primary astrocytes disrupted EAAT2 association with the lipid raft, resulting in loss of glutamate uptake function.[83] Under such circumstances, glutamate would accumulate in the intersynaptic space and enhance neural excitation through NMDARs and AMPARs, which can contribute to neurotoxicity (Figure 2). Inhibition of CH24H by soticlestat is hypothesized to preserve the membrane raft structure by inhibiting conversion of membrane cholesterol in the raft. Soticlestat thus maintains the association of EAAT2 with the raft microdomain, thereby

preserving glutamate reuptake from the synaptic cleft.[83] This is the second possible mechanism of action of soticlestat and is independent of the generation of the bioactive metabolite, 24HC.

7.3 | Link with neuroinflammation and glutamate regulation

It is widely acknowledged that inflammation in the brain significantly contributes to ictogenesis and epileptogenesis.[84,85] It has been proposed that seizures cause neuroinflammation that can exert impairment in astrocyte homeostatic functions, blood–brain barrier dysfunction, and excitation/inhibition imbalance, thereby resulting in neuronal circuit hyperexcitability and a reduced seizure threshold, evoking more seizures.[84] There seems to exist a vicious cycle centered around neuroinflammation. We observed a statistically significant correlation between brain levels of 24HC and tumor necrosis factor (TNF)-α in a soticlestat-treated PS19 tauopathy mouse model that exhibits hyperexcitability.[86] TNF-α is not only a master regulator of proinflammatory cytokine production, but is also known to regulate neuronal activity through multiple mechanisms.[87] Roles of TNF-α in epilepsy have been widely investigated and their complex association with seizures and epileptogenesis discussed.[88] Because of the vicious cycle of neuroinflammation in epilepsy, it was unclear whether the correlation between 24HC and TNF-α was a direct causal relationship or a consequence of tissue damage. In 2023, 24HC was reported to bind directly to αvβ3 integrin with high affinity ($K_D = 56.59\,\text{nmol}\cdot\text{L}^{-1}$) and trigger the production of proinflammatory cytokines such as TNF-α and interleukin-6 through activation of the focal adhesion kinase–nuclear factor κB signaling pathway.[55] In the same study, TNF-α was shown to induce expression of CH24H, suggesting another feedback loop among CH24H, 24HC, and TNF-α. Collectively, these observations suggest that soticlestat, by blocking the positive feedback loop, is expected to mitigate neuroinflammation and thereby ameliorate progression of epileptogenesis and ictogenesis (Figure 3).

Regarding the relevance to glutamate dysregulation, TNF-α is reported to trigger glutamate release from Bergman glia, therefore leading to an increase in the intrinsic excitability of Purkinje cells.[89] In addition, TNF-α exhibited concentration-dependent inhibition of glutamate uptake in hippocampal–entorhinal cortex brain slices, which then significantly increased and accelerated glutamate toxicity.[90] Together, TNF-α seems to act both to enhance release and to inhibit uptake of glutamate, hence contributing to neural overexcitation. The finding that soticlestat lowers 24HC concomitantly with TNF-α, likely

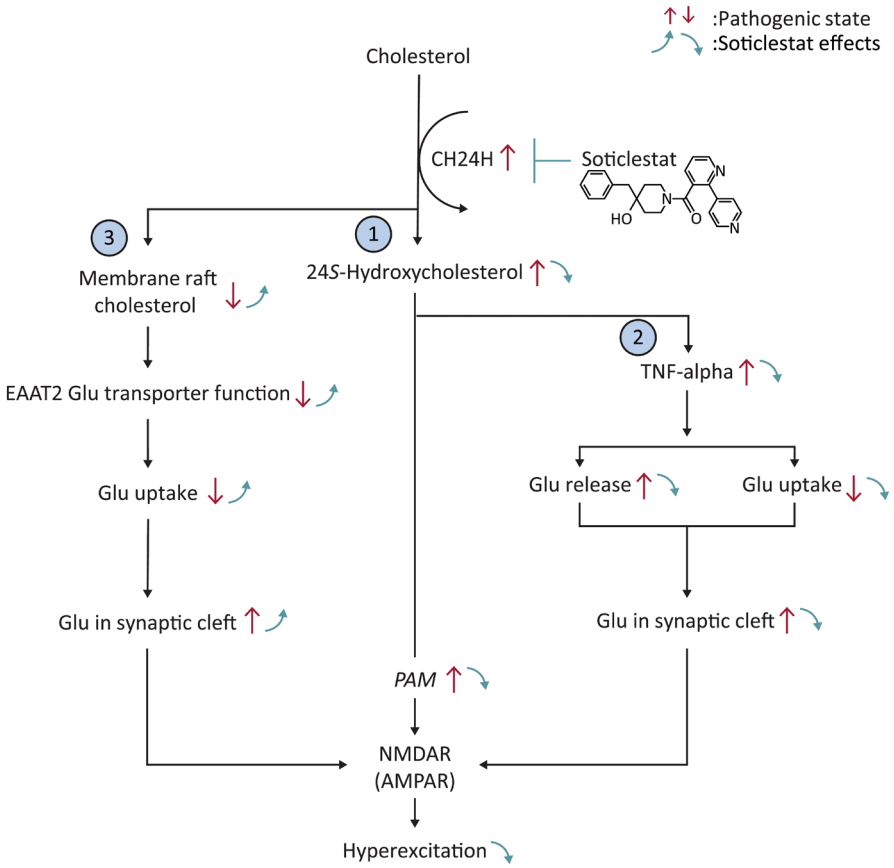


FIGURE 3 Purported soticlestat action on glutamate (Glu) hyperexcitation. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CH24H, cholesterol 24-hydroxylase (CYP46A1); EAAT2, excitatory amino acid transporter 2; NMDAR, N-methyl-D-aspartate receptor; PAM, positive allosteric modulator; TNF, tumor necrosis factor.

through the integrin-signaling pathway, presents unique and novel mechanisms for controlling chronic and progressive processes such as epileptogenesis.

The CH24H–24HC axis may also be linked with other inflammation-related pathways. The transcriptomic analysis in isolated neurons and astrocytes from the hippocampus of IAK mice developing epilepsy showed changes in signals in the glycoprotein IV-mediated activation cascade and the DAP12 pathway by soticlestat treatment.[26,84] Notably, genes in the glycoprotein IV-mediated activation cascade were differentially regulated in patients with tuberous sclerosis complex-associated epilepsy,[91] and proteins in the DAP12 signaling pathway were increased in microglia isolated from patients with mesial temporal lobe epilepsy.[92] Further studies are warranted for delineating roles of the CH24H–24HC axis in epileptic conditions associated with neuroinflammation.

8 | CONCLUSIONS

We described the roles of CH24H in brain homeostasis and epilepsy, and the discovery of a specific inhibitor, soticlestat, that has been investigated in phase III clinical trials as a potential treatment for Dravet syndrome and Lennox–Gastaut syndrome. The data support three potential mechanisms of action of soticlestat. First, reduction of 24HC that acts on NMDARs as a PAM is the most direct and selective mechanism targeting neurons. Second, restoring glutamate sequestration from the synaptic cleft preserves the membrane lipid raft microdomain, limits excessive glutamate excitation, and targets reactive astrocytes. Lipid raft preservation by inhibiting CH24H may possess broader functional consequences than just recovery of EAAT2 activity, because a wide array of membrane proteins gather in lipid rafts and transmit signals.[11] Lastly, by reducing 24HC and TNF-α, soticlestat possesses an ability to reduce neuroinflammation, effects that are likely to contribute to its antiseizure and other effects. For example, this mechanism of action seemingly has even broader implications as our understanding of neuroinflammation in epilepsy has evolved over the past two decades and revealed intriguing yet complex features involving multiple cell types in the brain.[93] These potential mechanisms of action, especially the second and third, warrant future studies to gain further insights into the antiseizure effects of soticlestat. Given that the majority of currently available antiseizure medications target both excitatory and inhibitory neurons, it is interesting to note that soticlestat likely targets neurons, astrocytes, and microglia that are all perturbed under disease conditions. What is clear is that soticlestat possesses a novel molecular mechanism of action relative to currently available antiseizure medications. Clinical application of soticlestat to acquired forms of epilepsy will depend on the findings of phase III clinical trials.

AUTHOR CONTRIBUTIONS

Shinichi Kondo: Conceptualization; visualization; original draft preparation. **Venkatesha Murthy**: Conceptualization; visualization; manuscript drafting and review. **Mahnaz Asgharnejad**: Conceptualization; visualization; manuscript drafting and review. **Arturo Benitez**: Conceptualization; writing (original draft preparation and review and editing); visualization. **Kosuke Nakashima**: Conceptualization; manuscript review. **Nicole Hawkins**: Writing (original draft preparation and review and editing). **H. Steve White**: Conceptualization; manuscript drafting, review and editing.

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CONFLICT OF INTEREST STATEMENT

S.K. and K.N. are employees of, and stockholders in, Takeda Pharmaceutical Company Limited. V.M., M.A., and A.B. are employees of Takeda Development Center Americas, Inc., and stockholders in Takeda Pharmaceutical Company Limited. N.H. and H.S.W. have served as consultants to Takeda.

DATA AVAILABILITY STATEMENT

Data compiled for this critical review are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Relevant details can be found in the original study publications. No additional human or animal research was performed specifically for reporting within this critical review. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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