DCP-LA, a New Strategy for Alzheimer’s Disease Therapy

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ABSTRACT

Alzheimer’s disease (AD) is characterized by extensive deposition of amyloid β (Aβ) and formation of neurofibrillary tangles (NFTs) consisting of hyperphosphorylated Tau. So far, a variety of AD drugs targeting Aβ have been developed, but ended in failure. A recent focus on AD therapy, therefore, is development of Tau-targeted drugs. Aβ activates glycogen synthase kinase-3β (GSK-3β), that plays a central role in Tau phosphorylation, responsible for NFT formation. The linoleic acid derivative DCP-LA has been developed as a promising drug for AD therapy. DCP-LA serves as a selective activator of PKCε and a potent inhibitor of protein tyrosine phosphatase 1B (PTP1B). DCP-LA restrains Tau phosphorylation efficiently due to PKCε-mediated direct inactivation of GSK-3β, to PKCε/Akt-mediated inactivation of GSK-3β, and to receptor tyrosine kinase/insulin receptor substrate 1/phosphoinositide 3-kinase/3-phosphoinositide-dependent protein kinase 1/Akt-mediated inactivation of GSK-3β in association with PTP1B inhibition. Moreover, DCP-LA ameliorates spatial learning and memory impairment in 5xFAD transgenic mice, an animal model of AD. Consequently, combination of PKCε activation and PTP1B inhibition must be an innovative strategy for AD therapy.

Introduction

Accumulating evidence has pointed to the role of amyloid β (Aβ), a main body of amyloid (senile) plaques, and Tau protein, a main body of neurofibrillary tangles (NFTs), in the pathogenesis of Alzheimer’s disease (AD). Huge studies have been done for development of AD drug targeting Aβ, but no expecting drug has been obtained. Recent target, therefore, has been turned to Tau.

Tau is abundantly expressed in neurons of the central nervous system and stabilizes microtubules by interacting with tubulin. Microtubules are the tracks for motor proteins bearing intracellular transport of vesicles, organelles and protein complexes,1-2, and Tau modulates microtubule dynamics including axonal transport3-6. Tau is upregulated during neuronal development, to promote generation of cell processes and establish cell polarity.7

When hyperphosphorylated, Tau detaches from the microtubules and forms fibrils in an insoluble form, referred to as paired helical filaments (PHFs), and NFTs comprises aggregation of PHFs.8-9 Tau is phosphorylated by a variety of serine/threonine protein kinases such as glycogen synthase kinase-3β (GSK-3β), cyclin-dependent kinase 5 (Cdk5)/p25, extracellular signal-regulated kinase 2 (ERK2), S6 kinase (S6K), microtubule affinity-regulating kinase (MARK), SAD kinase (SADK), protein kinase A (PKA), calcium/calcmodulin-dependent protein kinase II (CaMKII) or Src family kinases such as Fyn and c-Abl (Figure 1).10-14

Keywords
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Tau from the AD brain is phosphorylated at eleven Ser/Thr-Pro and nine Ser/Thr-X sites. Proline-directed kinases such as GSK-3β, Cdk5/p25, ERK2, and S6K and the non-proline-directed kinases MARK, SADK, PKA, and CaMKII phosphorylate Tau at the Ser/Thr residues. The non-receptor tyrosine kinases Fyn and c-Abl phosphorylate Tau at the tyrosine residues.

**Figure 1: Protein kinases relevant to Tau phosphorylation.**

The proline-directed kinases GSK-3β, Cdk5/p25, ERK2, and S6K and the non-proline-directed kinases MARK, SADK, PKA, and CaMKII phosphorylate Tau at the Ser/Thr residues. The non-receptor tyrosine kinases Fyn and c-Abl phosphorylate Tau at the tyrosine residues.

**Figure 2: GSK-3β plays a critical role in PHF-Tau phosphorylation.**

 Tau is initially phosphorylated by priming kinases such as non-proline-directed kinases (non-PDK). When GSK-3β activation is enhanced by Aβ, GSK-3β accelerates Tau-Ser396 phosphorylation, responsible for PHTs and NHFs, causing AD.

The serine/threonine protein kinases such as PKCε, Akt, PKA, integrin-linked kinase (ILK), CaMKII, p90 ribosomal protein S6 kinase (p90RSK), and protein kinase C-related kinase 2 (Prk2) inactivate GSK-3β by directly phosphorylating at Ser9 (Figure 3). Pyk2, that binds to SH2 and SH3 domain-containing proteins like Src kinases, and Fyn activate GSK-3β by phosphorylating at Tyr216 directly (Figure 3).

Akt is activated by being phosphorylated at Thr308 and Ser473 through the major pathway along a receptor tyrosine kinase (RTK)/insulin receptor substrate 1 (IRS-1)/PI3K/Akt axis. Then, Akt inactivates GSK-3β by phosphorylating at Ser9 and represses Tau phosphorylation.

GSK-3β is abundantly expressed in the brain, preferentially in the hippocampus. GSK-3β acts as the main executioner of Tau phosphorylation in PHFs. Intriguingly, GSK-3 accelerates the rate of Tau phosphorylation several-fold, if Tau is pre-phosphorylated by priming kinases such as non-proline-directed kinases. Of Tau phosphorylation sites, Ser396 phosphorylation is a key step in the PHF formation. Once a priming kinase phosphorylates Tau at Ser404, GSK-3β phosphorylates Tau at Ser400, followed by sequential phosphorylation of Ser396 (Figure 2). GSK-3β, alternatively, phosphorylates Tau at Ser202 directly, but Thr231 phosphorylation requires for Ser235 pre-phosphorylation.

**Interaction between Aβ and GSK-3β**

GSK-3β is originally in the active form. When phosphorylated at Ser9, GSK-3β is inactivated, but when phosphorylated at Tyr216, GSK-3β activation is enhanced. Aβ activates the non-receptor tyrosine kinase Fyn, to phosphorylate and activate GSK-3β, leading to somatodendritic accumulation of phosphorylated Tau. Aβ phosphorylates GSK-3β at Tyr216 and promotes Tau phosphorylation in PC-12 cells. Aβ, alternatively, activates GSK-3β by decreasing serine phosphorylation as a result of phosphoinositide 3-kinase (PI3K) inhibition/inactivation. Chronic exposure of Aβ downregulates Akt phosphorylation, to activate GSK-3β and increase Tau phosphorylation. Intracellular Aβ, on the other hand, promotes Tau phosphorylation and induces neuronal loss. GSK-3β exacerbates Aβ-induced neurotoxicity and cell death.

**Regulation of GSK-3β and Tau phosphorylation**

Amyloid precursor protein (APP) intracellular domain (AICD), that is produced from γ-secretase-mediated APP cleavage, activates GSK-3β or enters the nucleus and activates gene transcription, increasing the GSK-3β mRNA and protein. C-terminal fragments of APP stimulate GSK-3β activation, to increase Tau phosphorylation and induce apoptosis.

The interaction between Aβ and GSK-3β is further complicated by the regulation of Tau phosphorylation by other kinases and signaling pathways. For example, Akt phosphorylation inhibits GSK-3β activity, while GSK-3β itself can act as a negative regulator of Akt phosphorylation. These interactions highlight the complexity of the Tau phosphorylation process and the potential for therapeutic intervention.
insulin or insulin-like growth-factor 1 (IGF1) binds to and activates the RTK insulin receptor involving GSK-3β inactivation.

AMP-activated protein kinase (AMPK) is also shown to phosphorylate and inactivate GSK-3β. Pyk2 and Fyn phosphorylate GSK-3β at Tyr216 and activate GSK-3β.

Aging, inflammation, and stress activate GSK-3β, which triggers Tau phosphorylation, responsible for mild cognitive impairment (MCI), a preliminary group of AD (Figure 5). Aβ further activates GSK-3β and accelerates Tau phosphorylation, leading to progression into AD from MCI (Figure 5). Aggregation of hyperphosphorylated Tau causes tauopathies, a class of neurodegenerative diseases, that include frontotemporal dementia and parkinsonism linked to chromosome 17, progressive supranuclear palsy, Pick’s disease, and corticobasal degeneration as well as AD. Agents that have the potential to suppress GSK-3β activation, thus, could become beneficial preventive and therapeutic drugs for AD.

Tau-targeting drugs

Aβ and Tau serve as an initiator and an executor of AD, respectively. Current AD therapeutic approaches focus upon targeting Tau pathologies. A variety of Tau-targeting drugs have been developed as follows: i) Hsp90 inhibitors such as geldanamycin, radicicol, and 17AAG, that degrade and dispose of hyperphosphorylated Tau, ii) Inhibitors of Aβ-induced Tau phosphorylation such as kamikihito, DHA, and curcumin, iii) Tau aggregation inhibitors such as methylthioninium chloride and leucomethylthioninium, iv) O-GlcNAcase inhibitors, v) GSK-3β inhibitors such as pyrazine, the flavonoid morin, MMBO, the thiadiazolidinone derivative NP-12, and the traditional Chinese herbal medicine Angelica sinensis, vi) mTOR inhibitors, vii) Inhibitors of Tau fibrillization such as phenothiazine, the cyanine dye N744, polyphenol, porphyrin, anthracycles, phenylthiazolyl-hydrazide, PP2A axis.

Figure 3: Inactivation and activation of GSK-3β. PKCε, Akt, PKA, ILK, CaMKII, p90RSK, and Prk2 phosphorylate GSK-3β at Ser9 and inactivate GSK-3β. Pyk2 and Fyn phosphorylate GSK-3β at Tyr216 and activate GSK-3β.

Figure 4: RTK-mediated GSK-3β inactivation. Akt is activated through a pathway along a RTK/IRS-1/PI3K/PDK1/Akt axis and inactivate GSK-3β by phosphorylating at Ser9.

Figure 5: GSK-3β is a key factor for MCI and AD. Aging, inflammation, and stress activate GSK-3β and phosphorylate Tau, causing MCI. Aβ enhances GSK-3β activation and accelerates Tau phosphorylation, leading to progression into AD from MCI.
rhodanine, and aminothienopyridazines \(^{60,62}\), and viii) microtubule stabilizing agents including natural products such as taxanes, epothilones, discodermolide, dicytostatin, eleutherobin, sarcodyctins, laulimalide, peloraside A, cyclostreptin, tacalonolides, zampanolide, dactylolide, ceratamines, dicumarol, jatrophanes, tubercidin, lutein, and davunetide, and synthetic agents such as GS-164, estradiol analogues, 5HPP-33, triazolopyrimidines, pyridopyridazines, pyridotriazines, and pyridazines\(^{63-71}\). Successful results in the AD therapy, however, have not been obtained with any drugs as yet.

**8-[2-(2-Pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid (DCP-LA)**

Several lines of evidence have pointed to the role of cis-un saturated free fatty acids (uFFAs) such as arachidonic, linoleic, linolenic, oleic, and docosahexaenoic acid in cognitive functions\(^{63-71}\). Then, one would think that uFFAs might be available as an anti-dementia drug. uFFAs, however, are promptly metabolized and decomposed before arriving in the brain, even though orally or intravenously taken into the body. To address this issue, we have synthesized the linoleic acid derivative DCP-LA with cyclopropane rings instead of cis-double bonds, that exhibits stable bioactivities (Figure 6A,B)\(^{72}\).

DCP-LA induces a long-lasting facilitation of hippocampal synaptic transmission by enhancing presynaptic \(\alpha 7\) ACh receptor responses to stimulate glutamate release under the control of PKC\(\varepsilon\)\(^{72-75}\). In addition, DCP-LA activates CaMKII due to inhibition of protein phosphatase 1 (PP1), to enhance postsynaptic AMPA receptor responses and facilitate hippocampal synaptic transmission\(^{76}\).

The facilitatory action of DCP-LA on hippocampal synaptic transmission accounts for improvement of \(A_\beta\)\(^{40}\) and mutant \(A_\beta\)-induced spatial learning deficits in rats\(^{77,78}\), scopolamine-induced spatial learning and memory disorders in rats\(^{77}\), spatial learning and memory deterioration in senescence accelerated mice 8\(\{\text{SAMPB}\}\)\(^{79,80}\), and spatial learning and memory impairment in 5xFAD transgenic mice, an animal model of AD\(^{32}\).

PKC is classified into the conventional PKC isozymes \(\alpha, \beta, \beta II,\) and \(\gamma\), the novel PKC isozymes \(\delta, \epsilon, \eta,\) and \(\theta\), the atypical PKC isozymes \(\iota/\Lambda\) and \(\zeta\), and the PKC-like isozymes \(\mu\) and \(\nu\). All the PKCs have the phosphatidylserine (PS) binding site and are activated by diacylglycerol (DG). Much interestingly, DCP-LA is capable of selectively activating PKC\(\varepsilon\) in a Ca\(^{2+}\)- and DG-independent manner\(^{81}\). DCP-LA binds to the PS binding/associating sites Arg50 and Ile89 in the C2-like domain of PKC\(\varepsilon\) which are distinct from the DG binding site in the C1 domain, at the carboxyl-terminal end and the cyclopropane rings, respectively\(^{82}\).

DCP-LA efficiently inactivates GSK-3\(\beta\) and restrains Tau phosphorylation by cooperation of PKC\(\varepsilon\) activation and PTP1B inhibition

PKC\(\varepsilon\), activated by DCP-LA, inactivates GSK-3\(\beta\) by directly phosphorylating at Ser9 (Figure 6C). To develop DCP-LA as a medical drug, each diastereomer was separated and each characteristic was examined. Of 4 diastereomers \(\alpha,\beta\)-DCP-LA activates PKC\(\varepsilon\) selectively and stimulates presynaptic release of glutamate, dopamine, and serotonin, with the highest potency\(^{83}\). Of great interest is that DCP-PA serves as not only a selective PKC\(\varepsilon\) activator but a potent inhibitor of protein tyrosine phosphatase 1B (PTP1B). DCP-LA inhibits PTP1B by its direct interaction\(^{84}\).

![Figure 6: Structure of DCP-LA. DCP-LA has cyclopropane rings instead of cis-double bonds on linoleic acid (A,B). Racemic DCP-LA contains possible 4 diastereomers as \(\alpha,\alpha\)-, \(\alpha,\beta\)-, \(\beta,\alpha\)-, and \(\beta,\beta\)-DCP-LA (C).](Image.png)
PKCɛ activation or PTP1B inhibition, thus, has the potential to restrain Tau phosphorylation by inactivating GSK-3β independently. Cooperation of PKCɛ activation and PTP1B inhibition could inactivate GSK-3β and restrain Tau phosphorylation more efficiently than each solitary treatment. In experiments using PC-12 cells, PKCɛ overexpression and PTP1B deficiency activate Akt and inactivate GSK-3β synergistically. αβ_{1,42} activates GSK-3β by reducing Ser9 phosphorylation and increases Tau phosphorylation at Ser202/Thr205 and Ser396, and the effects of αβ_{1,42} are clearly neutralized by DCP-LA.

5xFAD mice are widely used as an animal model of AD. 5xFAD mice are APP/presenilin 1 (PS1) double transgenic mice that coexpress five familial forms of AD mutations such as the Swedish/London/Florida mutations and the M146L/L286V mutations. The αβ_{1,42} levels in the 5xFAD mouse brain increase in an age-dependent manner and spatial memory deficits are induced from 4-5 months of age. The significantly higher levels of GSK-3β-Ser9 phosphorylation are also found in the hippocampus of 5xFAD mice from 4-5 months of age as compared with the levels for wild-type control mice, indicating that the GSK-3β activity is enhanced in 5xFAD mice, possibly in association with αβ_{1,42} increase. Moreover, a greater deal of Tau-Ser396 phosphorylation, responsible for PHF formation, is found in the hippocampus of 5xFAD mice. GSK-3β suppression in the hippocampus of 5xFAD mice to an extent similar to that for wild-type control mice, DCP-LA, thus, enables efficient suppression of Tau-Ser396 hyperphosphorylation by activating PKCɛ and inhibiting PTP1B simultaneously.

DCP-LA ameliorates spatial learning and memory decline in 5xFAD mice, that occurs in parallel with GSK-β activation and an increase in Tau phosphorylation, but such effect is not obtained with galanthamine, that is clinically used for treatment of mild to moderate AD. In addition, DCP-LA improves αβ_{1,44} and mutant αβ-induced spatial learning deficits in rats, scopolamine-induced spatial learning and memory disorders in rats, spatial learning and memory deterioration in senescence accelerated mice. DCP-LA-induced improvement of cognitive decline is not due to only inhibition of GSK-β and restraint of Tau phosphorylation. Facilitation of synaptic transmission in alive neurons would be required for improvement of cognitive decline. DCP-LA has the potential to facilitate hippocampal synaptic transmission by enhancing presynaptic α7 ACh receptor responses under the control of PKCɛ and postsynaptic AMPA receptor responses under the control of CaMKII in association with PPI inhibition. This action of DCP-LA is also a strong advantage as an AD therapeutic drugs as compared with Tau-targeted drugs including GSK-β inhibitors. Tau-targeted drugs proposed possess no direct facilitatory action on synaptic transmission, and therefore, early improvement of cognitive decline would not be expected by those drugs.

A beneficial effect on 5xFAD mice is obtained with oral administration of DCP-LA at a dose of 1 mg/kg body weight, corresponding to ~3 μM. This dose, in the light of the fact that the optimal concentration of DCP-LA in the in vitro experiments is 100 nM, seems to be appropriate and possible for clinical use. Overall, DCP-LA may shed a beam of hope on AD prevention and treatment.

**Conclusion**

Tau-targeted drugs for AD therapy under development include i) Hsp90 inhibitors, ii) inhibitors of αβ-induced Tau phosphorylation, iii) Tau aggregation inhibitors, iv) O-GlcNAcase inhibitors, v) GSK-3β inhibitors, vi) mTOR inhibitors, vii) inhibitors of Tau fibrillization, and viii) microtubule stabilizing agents. The mechanism underlying the inhibitory effect of DCP-LA on Tau phosphorylation is distinct from that for any drugs provided until now. DCP-LA restrains Tau phosphorylation efficiently due to PKCɛ-mediated direct inactivation of GSK-3β, to PKCɛ/Akt-mediated inactivation of GSK-3β, and to RTK/IRS-1/P3K/PDK1/Akt pathway inactivation of GSK-3β in association with PTP1B inhibition. Consequently, combination of PKCɛ-mediated direct inactivation of GSK-3β, to PKCɛ/Akt-mediated inactivation of GSK-3β, and to RTK/IRS-1/P3K/PDK1/Akt pathway inactivation of GSK-3β in association with PTP1B inhibition.
activation and PTP1B inhibition must be an innovative strategy for AD therapy.

**Conflict of Interests Statement**

The author declares no conflict of interests.

**References**


