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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 transport[3-6]. 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/calmodulin-dependent protein kinase II (CaMKII) or Src family kinases such as Fyn and c-Abl (**Figure 1**)[10-14].

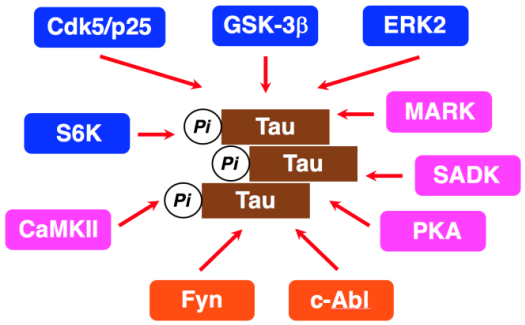

Figure 1: Protein kinases relevant to Tau phosphorylation. The proline-directed kinases GSK-3β, Cdk5/p25, ERK2, and S6K and the non-proline-directed kinases MARK, SADK, PKA, and CaMKII phosphorylate Tau at the Ser/Thr residues. The non-receptor tyrosine kinases Fyn and c-Alb phosphorylate Tau at the tyrosine residues.

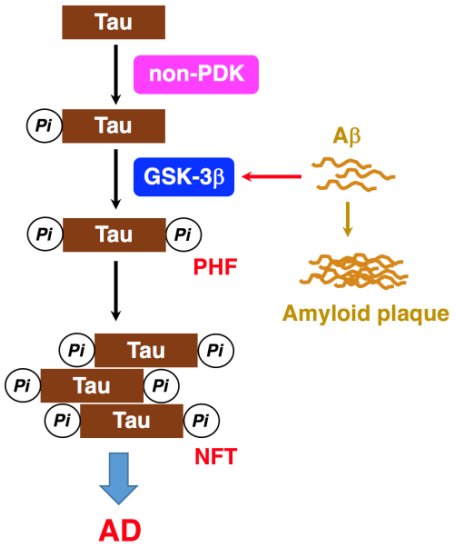

Figure 2: GSK-3β plays a critical role in PHF-Tau phosphorylation. Tau is initially phosphorylated by priming kinases such as non-proline-directed kinases (non-PDK). When GSK-3β activation is enhanced by Aβ, GSK-3β accelerates Tau-Ser396 phosphorylation, responsible for PHTs and NHFs, causing AD.

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 phosphorylate Tau at Thr181, Ser202/T205, Thr212/S214, Thr231/Ser235, and Ser396/Ser404 on Ser-Pro or Thr-Pro motifs in the regions flanking the repeat domains[10-12]. Non-proline-directed kinases such as MARK, SADK, PKA, and CaMKII phosphorylate Tau at Ser262, Ser 320, Ser324, and Ser356 on KXGS motifs in the repeat domains (R1-R4)[11,13,14]. Fyn and c-Abl, on the other hand, phosphorylate Tau at Tyr18 and Tyr394[11].

GSK-3β is abundantly expressed in the brain, preferentially in the hippocampus. GSK-3β acts as the main executioner of Tau phosphorylation in PHFs[15,16]. 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[17-19]. Of Tau phosphorylation sites, Ser396 phosphorylation is a key step in the PHF formation[20]. Once a priming kinase phosphorylates Tau at Ser404, GSK-3β phosphorylates Tau at Ser400, followed by sequential phosphorylation of Ser396 (**Figure 2**)[20]. GSK-3β, alternatively, phosphorylates Tau at Ser202 directly, but Thr231 phosphorylation requires for Ser235 pre-phosphorylation[20].

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[21].

Aβ activates the non-receptor tyrosine kinase Fyn, to phosphorylate and activates GSK-3β, leading to somatodendritic accumulation of phosphorylated Tau[22]. $A\beta_{1\text{-}42}$ phosphorylates GSK-3β at Tyr216 and promotes Tau phosphorylation in PC-12 cells[23]. Aβ, alternatively, activates GSK-3β by decreasing serine phosphorylation as a result of phosphoinositide 3-kinase (PI3K) inhibition/inactivation[24]. Chronic exposure of Aβ downregulates Akt phosphorylation, to activate GSK-3β and increase Tau phosphorylation[25]. Soluble Aβ oligomers inhibit insulin signaling relevant to Akt activation, to activate GSK-3β and increase Tau phosphorylation[26]. Intracellular $A\beta_{1\text{-}42}$ promotes Tau phosphorylation and induces neuronal loss[27]. GSK-3β exacerbates Aβ-induced neurotoxicity and cell death[28].

Amyloid precursor protein (APP) intracellular domain (AICD), that is produced from γ-secretase-mediated APP cleavage, activates GSK-3β [29] or enters the nucleus and activates gene transcription, increasing the GSK-3β mRNA and protein[30]. C-terminal fragments of APP stimulate GSK-3β activation, to increase Tau phosphorylation and induce apoptosis[31].

Regulation of GSK-3β and Tau phosphorylation

The serine/threonine protein kinases such as PKCε[32], Akt[32], PKA[33], integrin-linked kinase (ILK)[34], CaMKII[35], p90 ribosomal protein S6 kinase (p90RSK)[36], and protein kinase C-related kinase 2 (Prk2)[37] inactivate GSK-3β by directly phosphorylating at Ser9 (**Figure 3**). Pyk2[38], that binds to SH2 and SH3 domain-containing proteins like Src kinases, and Fyn[22] activate GSK-3β by phosphorylating at Tyr216 directly (**Figure 3**).

Akt1 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/3-phosphoinositide-dependent protein kinase 1 (PDK1)/Akt axis[32]. Then, Akt inactivates GSK-3β by phosphorylating at Ser9 and restrains Tau phosphoryaltion[32]. In the brain,

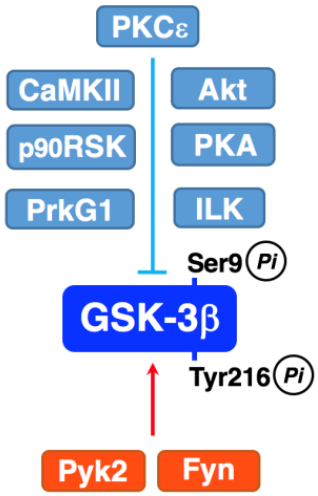

Figure 3: Inactivation and activation of GSK-3β. PKCε, Akt, PKA, ILK, CaMKII, p90RSK, and Prk2 phosphorylate GSK-3β at Ser9 and inactivate GSK-3β. Pyk2 and Fyn phosphorylate GSK-3β at Tyr216 and activate GSK-3β.

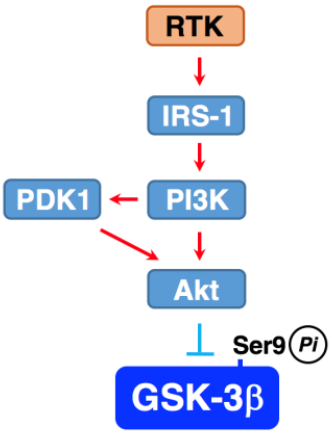

Figure 4: RTK-mediated GSK-3β inactivation. Akt is activated through a pathway along a RTK/IRS-1/PI3K/PDK1/Akt axis and inactivate GSK-3β by phosphorylating at Ser9.

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[39]. $A\beta_{1\text{-}42}$ upregulates expression of adenylate kinase-1 (AK1), to inhibit AMPK, thereby leading to GSK-3β activation and Tau phosphorylation[40]. A contradictory finding is that AMPK by itself phosphorylates Tau at Ser262 and induces tauopathy[41]. Moreover, a specific agonist of sphingosine-1-phosphate receptor 1 (S1PR1) linked to G_i protein reduces Tau-Ser262 phosphorylation in rat hippocampal slices[42]. This effect may be caused by AMPKα inactivation due to protein phosphatase 2A (PP2A) activation through a pathway along an S1PR1/G_i protein/(Cdc42/Rac1)/Pak1/PP2A axis.

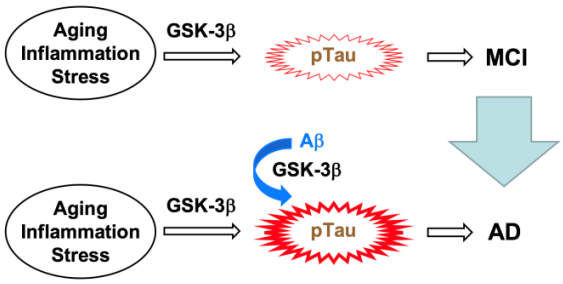

Figure 5: GSK-3β is a key factor for MCI and AD. Aging, inflammation, and stress activate GSK-3β and phosphorylate Tau, causing MCI. Aβ enhances GSK-3β activation and accelerates Tau phosphorylation, leading to progression into AD from MCI.

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**)[43,44]. 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[45]. 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[46], ii) Inhibitors of Aβ-induced Tau phosphorylation such as kamikihito, DHA, and curcumin[47,48], iii) Tau aggregation inhibitors such as methylthioninium chloride and leucomethylthioninium, iv) *O*-GlcNAcase inhibitors[49]. Tau is subjected to *O*-GlcNAc transferase-mediated *O*-GlcNAcylation at the Ser/Thr residues, that is the same sites as phosphorylation, and *O*-GlcNAcase neutralizes Tau *O*-GlcNAcylation. *O*-GlcNAcase inhibitors, therefore, promote Tau *O*-GlcNAcylation, thereby preventing Tau phosphorylation and aggregation[50], v) GSK-3β inhibitors such as pyrazine, the flavonoid morin, MMBO, the thiadiazolidinone derivative NP-12, and the traditional Chinese herbal medicine Angelica sinensis[51-55], vi) mTOR inhibitors[56,57]. Aβ activates mTOR, followed by activation of S6K, that phosphorylates Tau at Ser262, Ser214, and Thr212[12]. mTOR inhibitors, therefore, could prevent Tau phosphorylation, vii) Inhibitors of Tau fibrillization such as phenothiazine, the cyanine dye N744, polyphenol, porphyrin, anthracyclines, phenylthiazolyl-hydrazide,

rhodanine, and aminothienopyridazine[58,59], and viii) microtubule stabilizing agents including natural products such as taxanes, epothilones, discodermolide, dictyostatin, eleutherobin, sarcodyctins, laulimalide, peloruside A, cyclostreptin, taccalonolides, zampanolide, dactylolide, ceratamines, dicumarol, jatrophanes, tubercidin, lutein, and davunetide, and synthetic agents such as GS-164, estradiol analogues, 5HPP-33, triazolopyrimidines, phenylpyrimidines, pyridopyridazines, pyridotriazines, and pyridazines[60-62]. 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*-unsaturated 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 6 A,B**)[72].

DCP-LA induces a long-lasting facilitation of hippocampal synaptic transmission by enhancing presynaptic α7 ACh receptor responses to stimulate glutamate release under the control of PKCε[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$\beta_{1\text{-}40}$- and mutant Aβ-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 (SAMP8)[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 α, βI, βII, and γ, the novel PKC isozymes δ, ε, η, and θ, the atypical PKC isozymes ι/λ and ζ, and the PKC-like isozymes μ and ν. All the PKCs have the phosphatidylserine (PS) binding site and are activated by diacylglycerol (DG). Much interestingly, DCP-LA is capable of selectively activating PKCε 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 PKCε, which are distinct from the DG binding site in the C1 domain, at the carboxyl-terminal end and the cyclopropane rings, respectively[82].

Racemic DCP-LA contains possible 4 diastereomers such as α,α-, α,β-, β,α-, and β,β-DCP-LA (**Figure 6C**). To develop DCP-LA as a medical drug, each diastereomer was separated and each characteristic was examined. Of 4 diastereomers α,β-DCP-LA activates PKCε 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 PKCε activator but a potent inhibitor of protein tyrosine phosphatase 1B (PTP1B). DCP-LA inhibits PTP1B by its direct interaction[84].

DCP-LA efficiently inactivates GSK-3β and restrains Tau phosphorylation by cooperation of PKCε activation and PTP1B inhibition

PKCε, activated by DCP-LA, inactivates GSK-3β by directly phosphorylating at Ser9 (**Figure 6**)[32]. Activated PKCε, alternatively, activates Akt by directly phosphorylating at the serine residue, followed by inactivation of GSK-3β (**Figure 6**)[32].

When activated, RTK phosphorylates its own receptor at Tyr1185 and activates IRS-1 by phosphorylating at Tyr1222. Activated IRS-1 recruits and activates PI3K, which produces phosphatidylinositol 3,4,5-triphosphate (PIP_3)

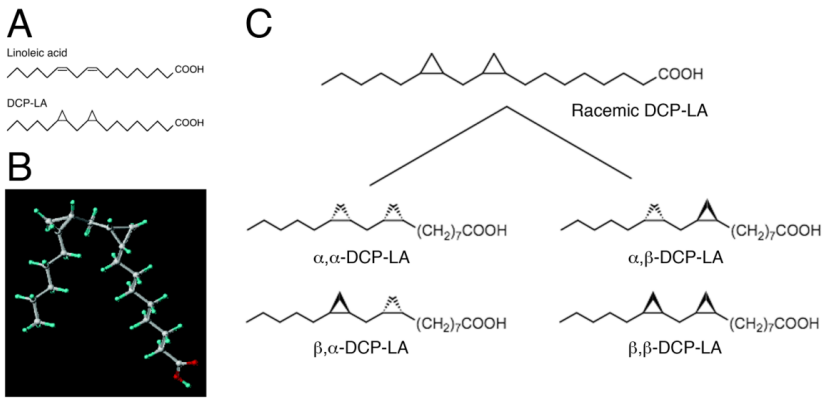

Figure 6: Structure of DCP-LA. DCP-LA has cyclopropane rings instead of *cis*-double bonds on linoleic acid (**A**,**B**). Racemic DCP-LA contains possible 4 diastereomers such as α,α-, α,β-, β,α-, and β,β-DCP-LA (**C**).

by phosphorylating phosphatidylinositol 4,5-bisphosphate (PIP_2). PIP_3 binds to and activates PDK1. PI3K and/or PDK1 activate Akt by phosphorylating at the serine and threonine residues. RTK and IRS-1 are inactivated through PTP1B-mediated tyrosine dephosphorylation. DCP-LA-induced PTP1B inhibition, therefore, represses inactivation of RTK and IRS-1, allowing Akt activation through a RTK/IRS-1/PI3K/PDK1/Akt pathway, to phosphorylate and inactivate GSK-3β (**Figure 6**)[32].

PKCε activation or PTP1B inhibition, thus, has the potential to restrain Tau phosphorylation by inactivating GSK-3β each independently. Cooperation of PKCε activation and PTP1B inhibition could inactivate GSK-3β and restrain Tau phosphorylation more efficiently than each solitary treatment[32]. In experiments using PC-12 cells, PKCε overexpression and PTP1B deficiency activate Akt and inactivate GSK-3β synergistically[32]. $A\beta_{1-42}$ activates GSK-3β by reducing Ser9 phosphorylation and increases Tau phosphorylation at Ser202/Thr205 and Ser396, and the effects of $A\beta_{1-42}$ are clearly neutralized by DCP-LA[32].

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[85]. The $A\beta_{1-42}$ levels in the 5xFAD mouse brain increase in an age-dependent manner and spatial memory deficits are induced from 4-5 months of age[85]. The significantly higher levels of GSK-3β-Ser9 phosphorylation is 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 $A\beta_{1-42}$ increase[86]. Moreover, a greater deal of Tau-Ser396 phosphorylation, responsible for PHF formation, is found in the hippocampus of 5xFAD mice[86]. DCP-LA suppresses GSK-3β activation and reduces Tau-Ser396 phosphorylation in the hippocampus of 5xFAD mice to an extent similar to that for wild-type control mice[32]. DCP-LA, thus, enables efficient suppression of Tau-Ser396 hyperphosphorylation by activating PKCε 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[32]. In addition, DCP-LA improves $A\beta_{1-40}$- and mutant Aβ-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[79,80]. 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 PKCε[72-75] and postsynaptic AMPA receptor responses under the control of CaMKII in association with PP1 inhibition[76]. This action of DCP-LA is also a strong advantage as an AD therapeutic drug 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.

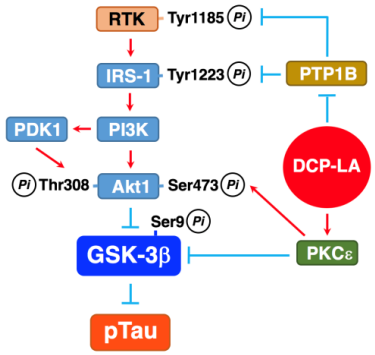

Figure 7: DCP-LA-induced suppression of Tau phosphorylation. PKCε, activated by DCP-LA, inactivates GSK-3β by phosphorylating Ser9 directly or through a PKCε/Akt pathway, to restrain Tau phosphorylation (pTau). DCP-LA-induced PTP1B inhibition, alternatively, activates Akt through a RTK/IRS-1/PI3K/PDK1/Akt pathway by repressing tyrosine dephosphorylation of RTK and IRS-1, followed by Ser9 phosphorylation and inactivation of GSK--β, to restrain Tau phosphorylation.

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 Aβ-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 PKCε-mediated direct inactivation of GSK-3β, to PKCε/Akt-mediated inactivation of GSK-3β, and to RTK/IRS-1/PI3K/PDK1/Akt-mediated inactivation of GSK-3β in association with PTP1B inhibition. Consequently, combination of PKCε

activation and PTP1B inhibition must be an innovative strategy for AD therapy.

Conflict of Interests Statement

The author declares no conflict of interests.

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