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 PKCe and a potent inhibitor of protein tyrosine phosphatase 1B (PTP1B). DCP-LA restrains Tau phosphorylation efficiently due to PKCe-mediated direct inactivation of GSK-3β, to PKCe/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 PKCe 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, and Tau modulates microtubule dynamics including axonal transport. Tau is upregulated during neuronal development, to promote generation of cell processes and establish cell polarity.

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. 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 (Figure 1).
Tau from the AD brain is phosphorylated at eleven Ser/Thr-Pro and nine Ser/Thr-X sites. Proline-directed kinases such as non-proline-directed kinases (non-PDK), 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.

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 activates GSK-3β, leading to somatodendritic accumulation of phosphorylated Tau. Aβ1-42 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 decreases insulin signaling relevant to Akt activation, to activate GSK-3β and increase Tau phosphorylation. Intracellular Aβ1-42 promotes Tau phosphorylation and induces neuronal loss. GSK-3β exacerbates Aβ-induced neurotoxicity and cell death.

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.

Regulation of GSK-3β and Tau phosphorylation

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) activate 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/3-phosphoinositide-dependent protein kinase 1 (PDK1)/Akt axis. Then, Akt inactivates GSK-3β by phosphorylating at Ser9 and restraints Tau phosphorylation. In the brain,
insulin or insulin-like growth-factor 1 (IGF1) binds to and activates the RTK insulin receptor involving GSK-3\(\beta\). Pyk2 and Fyn phosphorylate GSK-3\(\beta\) at Tyr216 and activate GSK-3\(\beta\).

Aging, inflammation, and stress activate GSK-3\(\beta\), which triggers Tau phosphorylation, responsible for mild cognitive impairment (MCI), a preliminary group of AD (Figure 5). A\(\beta\) further activates GSK-3\(\beta\) and accelerates Tau phosphorylation, leading to progression into AD from MCI.

**Figure 3:** Inactivation and activation of GSK-3\(\beta\). PKC\(\varepsilon\), Akt, PKA, ILK, CaMKII, p90RSK, and Prk2 phosphorylate GSK-3\(\beta\) at Ser9 and inactivate GSK-3\(\beta\). Pyk2 and Fyn phosphorylate GSK-3\(\beta\) at Tyr216 and activate GSK-3\(\beta\).

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

A\(\beta\) 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 A\(\beta\)-induced Tau phosphorylation such as kamikihito, DHA, and curcumin\(^{47,48}\), iii) Tau aggregation inhibitors such as methylthioninium chloride and leucomethylthioninium\(^{49}\), iv) O-GlcNAcase inhibitors\(^{50}\), v) GSK-3\(\beta\) 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\(^{56,57}\). A\(\beta\) activates mTOR, followed by activation of S6K, that phosphorylates Tau at Ser262, Ser214, and Thr212\(^{52}\). mTOR inhibitors, therefore, could prevent Tau phosphorylation, vii) Inhibitors of Tau fibrillization such as phenothiazine, the cyanine dye N744, polyphenol, porphyrin, anthracyclines, phenylthiazolyl-hydrazide, PP2A axis.
rhodanine, and aminothienopyridazine\textsuperscript{58,59}, and viii) microtubule stabilizing agents including natural products such as taxanes, epothilones, discodermolide, dicyostatin, eleutherobin, sarcodyctins, laulimalide, peloroside A, cyclosporin, 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\textsuperscript{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\textsuperscript{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, which exhibits stable bioactivities (Figure 6 A,B)\textsuperscript{72}.

DCP-LA induces a long-lasting facilitation of hippocampal synaptic transmission by enhancing presynaptic \(\alpha7\) ACh receptor responses to stimulate glutamate release under the control of PKC\(\varepsilon\)\textsuperscript{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\textsuperscript{76}.

The facilitatory action of DCP-LA on hippocampal synaptic transmission accounts for improvement of \(A\beta_{1-40}\) and mutant \(A\beta\)-induced spatial learning deficits in rats\textsuperscript{77,78}, scopolamine-induced spatial learning and memory disorders in rats\textsuperscript{77}, spatial learning and memory deterioration in senescence accelerated mice \textsuperscript{8}(SAMP8), and spatial learning and memory impairment in 5xFAD transgenic mice, an animal model of AD\textsuperscript{32}.

PKC is classified into the conventional PKC isozymes \(\alpha, \beta\), \(\beta\)II, and \(\gamma\), the novel PKC isozymes \(\delta, \epsilon, \eta, \theta, \) and the atypical PKC isozymes \(\iota/\lambda\) and \(\zeta\), and the PKC-like isozymes \(\mu\) and \(\nu\). All the PKCs have the phosphatidylycerine (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\textsuperscript{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\textsuperscript{82}.

Racemic DCP-LA contains possible 4 diastereomers such as \(\alpha, \alpha\), \(\alpha, \beta\), \(\beta, \alpha\), and \(\beta, \beta\)-DCP-LA (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\textsuperscript{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\textsuperscript{84}.

**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 6)\textsuperscript{22}. Activated PKC\(\varepsilon\), alternatively, activates Akt by directly phosphorylating at the serine residue, followed by inactivation of GSK-3\(\beta\) (Figure 6)\textsuperscript{32}.

When activated, RTK phosphorylates its own receptor at Tyr1185 and activates IRS-1 by phosphorylating at Tyr1185 and activates IRS-1 by phosphorylating at Tyr1185, followed by inactivation of GSK-3\(\beta\) at Tyr1222. Activated IRS-1 recruits and activates PI3K, which produces phosphatidylinositol 3,4,5-triphosphate (PIP\(_3\))
by phosphorylating phosphatidylinositol 4,5-bisphosphate (PIP₂). PIP₂ 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 treatment³². In experiments using PC-12 cells, PKCε overexpression and PTP1B deficiency activate Akt and inactivate GSK-3β synergistically³². Aβ₁₋₄₂ activates GSK-3β by reducing Ser9 phosphorylation and increases Tau phosphorylation at Ser202/Thr205 and Ser396, and the effects of Aβ₁₋₄₂ are clearly neutralized by DCP-LA³².

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 Aβ₁₋₄₂ 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 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 Aβ₁₋₄₂ increase. Moreover, a greater deal of Tau-Ser396 phosphorylation, responsible for PHF formation, is found in the hippocampus of 5xFAD mice. 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. 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-3β 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 Aβ₁₋₄₂- and mutant Aβ₁₋₄₂-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-3β 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 and postsynaptic AMPA receptor responses under the control of CaMKII in association with PP1 inhibition. This action of DCP-LA is also a strong advantage as an AD therapeutic drug as compared with Tau-targeted drugs including GSK-3β 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 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.

References

32. Shelly M, Lim BK, Cancello DA, et al. Local and long-range reciprocal regulation of cAMP and cGMP in axon/dendrite formation. Science. 2010; 327(5965): 547-552.

38. Sayas CL, Ariasens A, Ponsens B, et al. GSK-3 is activated by
the tyrosine kinase Pyk2 during LPA1-mediated neurite retraction. Mol

nectaridin B, a nutmeg lignan, against oxidative stress: Role of Nrf2
activation through ERK phosphorylation and AMPK-dependent

kinase-1 in Aβ-mediated tau phosphorylation via AMPK and GSK3β.

regulatory pathway in tau-mediated toxicity. Biol Open. 2017; pii:
bio.022863.

42. St-Cyr Gigaère F, Attioli Esis S, Chagniel L, et al. The sphingosine-1
phosphate receptor 1 agonist SR12271 reduces TauSer262
phosphorylation in rat hippocampal slices. Brain Res. 2017; 1658:
51-59.

43. Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration
8(9): 663-672.

44. Hurtado DE, Molina-Porcel L, Iba M, et al. Aβ accelerates the
spatiotemporal progression of tau pathology and augments tau

45. Stancu IC, Ris L, Vasconcelos B, et al. Tauopathy contributes to
synaptic and cognitive deficits in a murine model for Alzheimer’s

misfolded tau protein induced by geldanamycin is associated with
339-348.

47. Watari H, Shimada Y, Tohda C. New treatment for Alzheimer’s
disease, kamihiko, reverses amyloid-β-induced progression of
tau phosphorylation and axonal atrophy. Evid Based Complement

48. Ma QY, Yang F, Rosario ER, et al. β-amyloid oligomers induce
phosphorylation of tau and inactivation of insulin receptor substrate
via c-Jun N-terminal kinase signaling; suppression by omega-3 fatty

49. WischikCM, Harrington CR, Storey JM. Tau-aggregation inhibitor therapy

pathological tau without affecting its normal phosphorylation in a
mouse model of tauopathy. Neuropharmacology. 2014; 79:
307-313.

highly selective glycogen synthase kinase-3 (GSK3β) inhibitors
for Alzheimer’s disease: design, synthesis, and characterization of

52. Gong EJ, Park HR, Kim ME, et al. Morin attenuates tau
hyperphosphorylation by inhibiting GSK3β. Neurobiol Dis. 2011;

kinase-3 inhibitor 2-methyl-5-[3-(4-[5]-methylsulfonylphenyl)-1-
benzoxa-6-yl]-1,3,4-oxadiazole decreases tau phosphorylation
and ameliorates cognitive deficits in a transgenic model of Alzheimer’s

reduces Alzheimer’s pathology and rescues neuronal loss in vivo.

55. Zhang Z, Zhao R, Qi J, et al. Inhibition of glycogen synthase kinase-3β by
Angelica sinensis extract decreases β-amyloid-induced neurotoxicity
2011; 89(3): 447-447.

56. Cacchione A, Magri A, Medina DX, et al. mTOR regulates tau
phosphorylation and degradation: implications for Alzheimer’s

between mammalian target of rapamycin (mTOR), amyloid-β, and
13107-13120.

penetrant, orally bioavailable aminothienopyridazine inhibitors of

agents as potential treatment for Alzheimer’s disease and related
8979-8996.

60. Brunden KR, Ballatore C, Lee VM, et al. Brain-penetrant microtubule-
stabilizing compounds as potential therapeutic agents for tauopathies.

agent, epothilone D, reduces axonal dysfunction, neurotoxicity,
cognitive deficits, and Alzheimer-like pathology in an intervention
3610-3611.

microtubule-stabilizing drugs as possible therapeutic agents for
2011; 63(3): 341-351.

ACh receptor currents by protein kinase C activation but not by
receptor phosphorylation. Biochem Biophys Res Commun. 1996;
221(3): 716-721.

64. Nishizaki T, Matsuoka T, Nomura T, et al. Modulation of ACh receptor
currents by arachidonic acid. Mol Brain Res. 1998; 57(1):
173-179.

messenger for the expression of long-term potentiation. Biochem

currents through Ca2+-permeable AMPA receptors by interacting with

a long-lasting facilitation of hippocampal synaptic transmission by
modulating PKC activity and nicotinic ACh receptors. Mol Brain Res.

68. Nishizaki T, Ikeuchi Y, Matsuoka T, et al. Short-term depression and
long-term enhancement of ACh-gated channel currents induced by

hippocampal neurotransmission via a phospholipase A2 signaling

70. Nishizaki T, Ikeuchi Y, Matsuoka T, et al. Oleic acid enhances ACh
receptor currents by activation of Ca2+/calmodulin-dependent protein

fatty acids on PKC-ε activation and nicotinic ACh receptor responses.

72. Tanaka A, Nishizaki T. The newly synthesized linoleic acid derivative
FR236924 induces a long-lasting facilitation of hippocampal
neurotransmission by targeting nicotinic acetylcholine receptors.


