PERSPECTIVE

Okadaic acid: a tool to study regulatory mechanisms for neurodegeneration and regeneration in Alzheimer's disease

Okadaic acid: Okadaic acid (OKA), a polyether (C38 fatty acid) toxin, is a potent and selective inhibitor of protein phosphatase, PP1 and protein phosphatase 2A (PP2A). It is mainly extracted from a black sponge *Hallichondria okadaii* and has been suggested to play a potent probe for studying the various molecular, cellular, biochemical and mechanism of neurotoxicity. It is known as a selective and potent inhibitor of serine/threonine phosphatases 1 and 2A induces hyperphosphorylation of tau *in vitro* and *in vivo*. It has been reported that Alzheimer's disease (AD) is a complex multifactorial neurodegenerative disorder and hyperphosphorylated tau protein is a major pathological hallmark of AD. The reduced activity of phosphatases like, PP2A has been implicated in the brain of AD patients. OKA also induced inhibition of protein phosphatases cause neurofibrillary tangles (NFTs) like pathological changes and tau hyperphosphorylation seen in AD pathology. Our and others reports inferred that OKA induces neurodegeneration along with tau hyperphosphorylation, GSK3β activation, oxidative stress, neuroinflammation and neurotoxicity which are characteristic of AD pathology (**Figure 1**).

Alzheimer's disease (AD): AD is pathologically characterized by deposition of extracellular amyloid beta (Aβ) plaques and intracellular NFTs (Najem et al., 2014). NFTs are composed of bundles of paired helical filaments (PHFs) and conservative filaments which are the major protein components of the abnormally hyperphosphorylated tau protein. Tau is a major phosphoprotein in central nervous system which usually containing two to three phosphate groups per molecule. Abnormal hyperphosphorylation of tau is believed to be responsible for neuronal toxicity and loss of neuronal activity which further promotes aggregation of PHFs (Murray et al., 2013). Hence, the abnormal hyperphosphorylation of tau appears to be critical to the pathogenesis of AD. Tau phosphorylation is usually catalyzed by tau protein kinases and reversed by tau protein phosphatases (PPs). There are many phosphatases expressed in brain and among them PP2A is highly expressed. OKA has potential properties to inhibit PP2A activity thereby causing tau hyperphosphorylation. It has been reported that the expressions and activities of some phosphatases are decreased in the affected areas of AD brain. Our observations from several studies suggest that a down regulation of tau phosphatases in AD brain might trigger the abnormal hyperphosphorylation of tau and other neuronal proteins. Interestingly, OKA showed similar kind of properties in various studies.

Okadaic acid and kinases: Recent studies have also emphasized the importance of serine/threonine protein phosphatases in many neurodegenerative processes including neuronal apoptosis. The serine/threonine protein phosphatases are also associated with activation of major kinases such as Ser/Thr, MAPK, ERK, PKA, JNK, PKC and GSK3β in neurons. These enzymes and signaling molecules are actively associated with AD pathology. These kinases promote abnormal hyperphosphorylation of tau, suggest that the cascade of these kinases could exclusively be involved in the pathogenesis of AD. The activity of serine/threonine protein phosphatases needs extensive study and OKA is a very important and suggestive tool for this purpose. These enzymes are potential targets for many diseases, including inflammatory and neurodegenerative diseases. Reports from a previous study suggested that OKA may have rapid metabolic consequences on cell death by altering rates of phosphorylation-dephosphorylation *in vivo*. OKA leads to β-amyloid deposition and subsequent neuronal degeneration, synaptic loss, and memory impairments, all of these are characteristic features of AD progression (Kamat, 2013, 2014). Cognitive deficiency/dementia or cognitive functional declines are the ultimate outcomes in AD patients. Our lab reports have shown that the microinfusion/intracebroventricular/intracerebral injection of OKA into the rodent's brain causes cognitive deficiency by β-amyloid deposition and tau hyperphosphorylation. The phosphorylation state of proteins modulated by protein kinases and protein phosphatases is important in the regulation of physiological processes. In the central nervous system, protein phosphorylation plays a critical role in the molecular mechanisms through which neurotransmitters and hormones produce their biological effects in target cells (Duan et al., 2013). Alteration of the normal rates of phosphorylation and dephosphorylation of proteins may affect neuronal functions. There are some evidences which support that protein kinases and phosphatases are involved in the cascade of events that contribute to neuronal toxicity under pathological conditions (Crespo-Biel et al., 2013). The neurotoxicity which is associated with an abnormally phosphorylated form of the microtubule-associated protein (MAP) tau promotes AD-like pathology. Tau protein is a major constituent of the paired helical filaments, characteristic of the AD and their aberrant protein phosphorylation has also been implicated in the processing and secretion of amyloid peptides whose extracellular deposition forms the senile plaques (Tamaoka et al., 2013). In clinical set of experiment; it has been observed that, in AD brain, the activity of PP2A appears to be reduced in both gray and white matters and a down-regulation of this enzyme is apparently involved in slowing down the process of tau dephosphorylation. Several studies have suggested the significance of MAPK cascade in mammalian synaptic plasticity, learning, and memory (Sharma et al., 2004). MAPK cascade which is strongly associated with oxidative stress during AD pathology and other neurological disorder was originally discovered as a critical regulator of cell division and differentiation. In later stage, it has been also shown that extracellular receptor

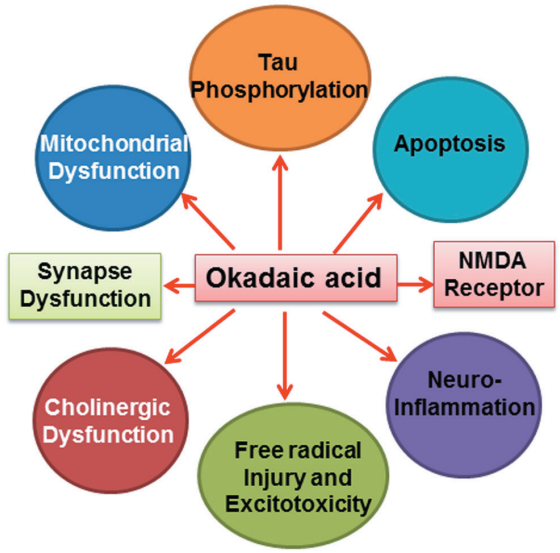

Figure 1 Cartoon represents the effects of okadaic acid (OKA) on various biochemical and molecular events such as tau hyperphosphorylation, apoptosis, synapse dysfunction, cholinergic dysfunction, free radical injury and neuroinflammation which are contributory factors in AD pathogenesis.
AD: Alzheimer's disease; NMDA: N-methyl-D-aspartate.

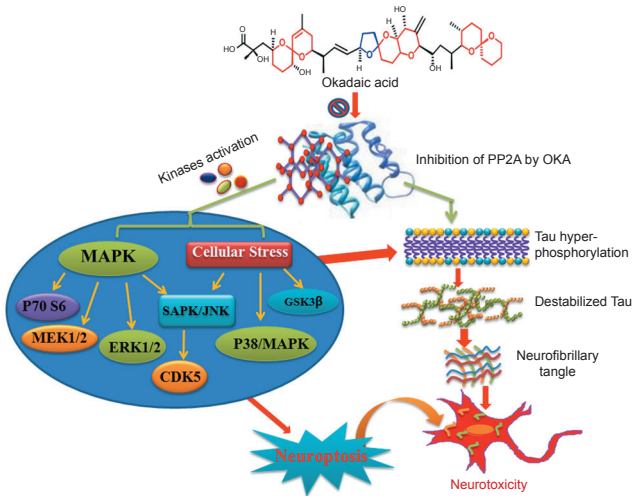

Figure 2 Flow diagram depicts the possible mechanism of okadaic acid (OKA) induced neurotoxicity by various protein kinases associated with phosphatases activity. These kinases are regulating and interacting with each other during AD pathogenesis. PP2A inhibition by OKA induces kinases activity which promotes cellular oxidative stress and activation of major kinases, for example, MAPK and ERKs associated with AD progression.
AD: Alzheimer's disease; PP2A: protein phosphatase 2A; MAPK: mitogen-activated protein kinase; ERK: extracellular regulated kinases; CDK5: cyclin dependent kinase 5; GSK3β: glycogen synthase kinase 3 beta; SAPK/JNK: stress-activated protein kinase/c-Jun N-terminal kinase; MEK1/2: mitogen-activated protein kinase kinase 1/2.

kinase (ERKs) is abundantly expressed in neurons of mature central nervous system and associated with regeneration of neurons. The ability of ERK1/2 to phosphorylate tau *in vitro* at many abnormal hyperphosphorylation sites is observed in AD brain (Toyoda et al., 2007). Kadish and van Groen (2014) reported that lesions of the entorhinal cortex in normal, aged, and transgenic mice produce different responses. Aged mice showing less regeneration after lesion and the presence of Aβ deposits in transgenic mice does not prevent post-lesion propagation. They observe increased regeneration in presenilin mutant mice but no increased regeneration in mice overexpressing Aβ leading them to conclude that the presence of Aβ does not prevent attempts at neuronal repair. Since OKA produces similar condition of Aβ deposition *in vivo* and *in vitro*; therefore, OKA also can be used to study the neuronal regeneration or degeneration mechanism.

Pathological events in AD progression: AD progression is commonly believed that the pathologically accumulated cellular products such as Aβ, phospho-tau, proinflammatory molecules cause neuronal and synaptic degeneration (Kamat et al., 2014). These molecules are largely related to anabolic and biosynthetic pathways mediating cell growth/differentiation, neuritic extension, synaptic plasticity, cell adhesion, and cytoskeleton and signaling control for the above neuronal processes. Alternatively, AD may be also considered as a metabolic disease that is primarily caused by bioenergetics failure ie mitochondrial dysfunction. Bioenergetics failure by the progressive effects of age related damage (oxidative damage) with a compensatory regulatory mechanism of energy metabolism occurs by affected neurons. An overall reduced oxidative bioenergetics metabolism in the brain during normal aging, and in AD patients, especially in cerebral regions critically involved in cognitive utility. A recent microarray analyses have clearly demonstrated prominent upregulation of numerous genes associated with biosynthetic cellular events (transcription, protein biosynthesis, protein trafficking, and turnover), mitochondrial energy generation, as well as synaptic function maintenance in the brains of AD patients. However, little is known about the cellular or molecular mechanism; how the rate of phosphorylation/ dephosphorylating of proteins in the central nervous system could be related with normal or altered cellular functions such as; neuronal function, synaptic function, and mitochondrial function. Although these experimental paradigms are not sufficient to fully recapitulate the AD pathology. OKA provides a potentially useful tool for studying the participation of gene regulatory mechanism of tau hyperphosphorylation and neuronal death processes. In our ongoing studies, our lab focuses on the effects and mechanism of OKA on synapse dysfunction. Since the synapse dysfunction is implicated in AD pathology and synapse plasticity is necessary for normal neuronal functions. We are basically targeting the tau proteins and trying to elucidate their mechanism on synapse dysfunction.

Protein phosphorylation represents wider implication in learning and memory; therefore, OKA may be the suitable alternative model to understand the mechanism underlying the process of neurodegeneration linked with memory deficit and AD pathology. Collective evidence from several studies shows that inhibition of phosphatase (PP-2A) activity leads to hyperphosphorylation of tau, not only due to a reduction in its dephosphorylation but probably also by

phosphorylation and activation of kinases (**Figure 2**). OKA also leads to phosphorylation of tau and GSK3β by inhibition of PP2A activity and thereby extracellular deposition of β-amyloid in senile plaques, intracellular formation of NFT (consisting a phosphorylated form of a microtubule associated protein, tau), and the loss of neuronal synapses and pyramidal neurons and builds up a similar condition like clinical neuropathology of AD. Thus, OKA induced PP2A gene regulatory mechanism and kinases remodeling play a crucial role in the abnormal hyperphosphorylation of tau which results in neurotoxicity and build up similar condition of AD pathology.

Advantages of OKA to study neurodegeneration: OKA-induced neurodegeneration produced a similar characteristic pathology such as tau hyperphosphorylation, NFT formation, GSK3β activation and other molecular changes like cholinergic dysfunction, astroglial activation, neuroinflammation, mitochondrial dysfunction, decreased cerebral blood flow and memory impairment (Rajasekar et al., 2013; Kamat et al., 2012). On the other hand, OKA induces neurodegeneration and activates most of the kinases involved in AD pathology.

Precaution for using OKA to study neurodegeneration: OKA is a chemical which is used to study AD pathology and regulatory mechanism for neurodegeneration. Since, OKA is available in the market in different preparations of the compound or salt such as ammonium salt, potassium salt, sodium salt, *etc.* Somehow the protein phosphatase 2A (PP2A) inhibition by OKA at the same concentration varies. Reports suggested that there are considerable differences in toxicity at the same concentration of different salts or compound of OKA. PP2A inhibition is an important phenomenon for tau hyperphosphorylation, which is one of the main pathological events for AD. So, we recommend that before using any salt or compound of OKA, it is necessary to know what amount of OKA inhibits the what extent of the PP2A activity.

Key findings in AD by utilizing OKA as a tool: By utilizing OKA as a tool for AD model, we and others have found that OKA produces oxidative stress, mitochondrial dysfunction, cholinergic dysfunction altered NMDARs function, apoptotic cell death, neuroinflammation and neurotoxicity. All these altered functions are contributory factors for development of AD-like pathology. On the other hand, protection offered by clinically used anti-AD drugs (memantine, donepezil) shows disorder like AD. Therefore, OKA can be used as an experimental tool to study mechanisms of neurodegeneration and unravel novel therapeutics targets for AD pathology.

Concluding remarks: Evidence from previous reports indicates that serine/threonine protein phosphatases and kinases are potentially used as therapeutic targets in several diseases. Hence, from a translational point of view, OKA induced neurodegeneration model may also be capable to detect the drug acting simultaneously on protein phosphorylation and dephsophorylation process.

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