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Effect of pesticides on phosphorylation of Tau protein, and its influence on Alzheimer's disease

Torres-Sánchez ED *et al*. Pesticides on phosphorylation of Tau protein

Abstract

Alzheimer's disease (AD) is a progressive and neurodegenerative illness which results in alterations in cognitive development. It is characterized by loss/dysfunction of cholinergic neurons, and formation of amyloid plaques, and formation of neurofibrillary tangles, among other changes, due to hyperphosphorylation of tau-protein. Exposure to pesticides in humans occurs frequently due to contact with contaminated food, water, or particles. Organochlorines, organophosphates, carbamates, pyrethroids and neonicotinoids are associated with the most diagnosed incidents of severe cognitive impairment. The aim of this study was to determine the effects of these pesticides on the phosphorylation of tau protein, and its cognitive implications in the development of AD. It was found that exposure to pesticides increased the phosphorylation of tau protein at sites Ser198, Ser199, Ser202, Thr205, Ser396 and Ser404. Contact with these chemicals altered the enzymatic activities of cyclin-dependent kinase 5 and glycogen synthase kinase 3 beta, and protein phosphatase-2A. Moreover, it altered the expression of the microtubule associated protein tau gene, and changed levels of intracellular calcium. These changes affected tau protein phosphorylation and neuroinflammation, and also increased oxidative stress. In addition, the exposed subjects had poor level of performance in tests that involved evaluation of novelty, as test on verbal, non-verbal, spatial memory, attention, and problem-solving skills.

Key Words: Organochlorines; Organophosphates; Carbamates; Pyrethroids; Neonicotinoids; Tau protein

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Core Tip: Exposure to pesticides occurs frequently through contact with contaminated particles, food, or water. In 2022, the Alzheimer's Association emphasized that contact with these pollutants is a risk factor for Alzheimer diseases. This study showed that contact with organochlorines, organophosphates, carbamates, pyrethroids and neonicotinoids modified mechanisms related to tau hyperphosphorylation and neuroinflammation. In cognitive findings, these chemicals altered memory, attention, and problem-solving processes. Few published studies have evaluated the effect of these pesticides on tau protein. Therefore, this review is novel in the sense that it presents an analysis for each pesticide class.

INTRODUCTION

Alzheimer's disease (AD) represents 60%-70% of the cases of major cognitive disorders (MCD) worldwide. In 2022, data from the World Health Organization[1] showed that approximately 55 million people were diagnosed with MCD. Additional data from Alzheimer's Disease International indicate that by 2030, this figure may increase to 78 million, with most cases expected to come from developing countries[2]. Amongst the risk factors associated with the development and progression of AD, exposure to pollutants has been linked to poor cognitive impairment[3]. These pollutants comprise organochlorine (OCs) pesticides, organophosphates (OPs), carbamates (Cs), pyrethroids (Ps) and neonicotinoid insecticides (Ns). Majority of them have neurotoxic potential. Environmental pollution by pesticides occurs through airborne dust particles, resulting in frequent contamination of air, water and food. Approximately, 30% of these chemicals are dispersed in powder form, while the remaining 70% are volatilized into the environment from surfaces where they are applied[4-7]. Another indirect form of contact with these chemicals is through consumption of contaminated water and food. Previous studies from India, Brazil, Lithuania, Egypt, Turkey, Mexico, and Venezuela revealed presence of contamination in vegetables, fruits, cereals, and water *via* exposure to OCs, OPs, Cs, Ps and Ns[5,8-10].

Pesticides are organic and hydrophobic molecules which are easily absorbed through different routes of exposure in humans. Pesticides are distributed mainly in lipid tissues of the body where they bioaccumulate as residues[5,11]. It is important to note that the brain and central nervous system (CNS) are rich in lipids, mainly sphingolipids, cholesterol, glycerophospholipids and omega-3 and omega-6 polyunsaturated fatty acids[12]. Thus, the brain and CNS are anatomical sites vulnerable to pesticides due to physicochemical affinity[5,11]. Previous studies indicate that in CNS, exposure to pesticides alters neurogenesis and leads to cognitive impairment[13,14]. However, there is still doubt about the involvement pesticides in the development of AD, and the underlying pathophysiological mechanisms. Most of the published studies on patients with AD or in experimental models were focused mainly on evaluation of the effects of exposure to two classes of pesticides: OC and OP. There are limited reports on effect of exposure to Cs, Ps and Ns, and the impact of the pesticides on tau protein phosphorylation. Tau protein, which is expressed in the distal extremity of the axon, controls the stability of microtubules. Hyperphosphorylation of tau protein stimulates the dissociation of microtubules, interrupts axonal extension, and enhances the aggregation of insoluble tau, leading to alterations in the synapse, and hence tauopathy[15,16]. Therefore, the present this study was aimed at investigating the effects of OC, OP, C, P and N pesticides on the phosphorylation of tau protein, and the associated cognitive implications in the development of AD.

PESTICIDES AND CNS EFFECTS

In a general way, the major reported effects of pesticide exposure on CNS are changes in enzymatic activity of acetylcholinesterase (AchE), blockage of receptors, blockage of transport channels, changes in steroidal hormonal responses, mitochondrial damage, and increased oxidative stress, all of which affect motor, sensory, autonomous, and cognitive functions[14,17,18]. Specifically, OC pesticides block calcium-dependent sodium-potassium pump and chloride channels, a phenomenon that generates antagonistic effect on the neurotransmitter gamma aminobutyric acid (GABA), leading to increases

in CNS excitotoxicity[4,17,19]. On the other hand, OP and C pesticides inhibit AchE: OPs bind irreversibly to the active site of AchE, while Cs binds reversibly to AchE[4,17,18]. The inhibition of AchE increases the concentration of acetylcholine, thereby overstimulating postsynaptic muscarinic receptors[4,19,20]. Therefore, exposures to OP and C pesticides have been linked to the development of MCD through changes in acetylcholine levels[4]. The class P pesticides act *via* 3 different mechanisms. Firstly, they bind to voltage-dependent sodium channels, thereby modifying their conformations. This affects the transition from ion to non-conductive state which results in a higher sodium input. Secondly, they block the binding of calcium to calmodulin, resulting in increases in calcium ion concentration which alter the neurotransmission and depolarization of the N-methyl-D-aspartate receptor. Thirdly, they bind to chloride-dependent GABA receptors. These 3 mechanisms alter muscarinic, adrenergic, and serotonergic neurotransmissions, resulting in symptoms such as tremor, prostration, sensitivity to stimuli, choreoathetosis, salivation and clonic seizures[4,17,21]. The N pesticides are nicotinic receptor agonists in CNS postsynaptic neurons. The nicotinic receptors are part of important ion channels in the neurotransmission functions of acetylcholine, GABA, glycine, and glutamate. Therefore, exposure to N pesticides triggers nicotinic syndrome which involves the respiratory and cardiovascular functions, as well as CNS[4,17,22].

Other disturbances induced by pesticide exposure are linked to estrogen steroid receptors and steroid nuclear receptors. Interactions of pesticides with these receptors alter various metabolic and genetic pathways, which may lead to multiple pathologies, including AD[18,23]. It has been reported that several pesticides act as endococcal disruptors due to their ability to inhibit cytochrome P450 enzyme complex in the brain, with adverse impact on the synthesis of steroid hormones, vitamins, retinoic acid, and thyroid hormones[18]. It has been reported that exposure to hydroxychlor, an OC pesticide, resulted in antagonism of estrogen receptors alpha and beta, a situation which may lead to neurological alterations[24]. Besides, due to their hydrophobic characteristics, the OC and OP pesticides are easily incorporated into the mitochondria

along with mitochondrial respiratory chain translocating proteins, leading to enhanced oxidative damage, increased mitochondrial permeability, induction of apoptosis, and decreased synthesis of ATP[25].

Additionally, pesticide-induced overactivation of cholinergic/glutamatergic responses and increased concentration of intracellular calcium ion, are associated with increased free radicals, mainly reactive nitrogen species and reactive oxygen species, thereby tilting the oxidant-antioxidant balance towards pro-oxidants[25,26]. A break in the mitochondrial oxidant-antioxidant homoeostasis results in loss of neuronal synapses, as well as neuropathological, neurochemical, and neurobehavioral alterations[4].

PESTICIDES AND THEIR IMPACTS ON TAU PROTEIN

Tau protein and tauopathies

AD is characterized by loss of cholinergic neurons or dysfunctional cholinergic neurons, formation of amyloid plaques, and formation of neurofibrillary tangles, due to hyperphosphorylation of tau-protein[15,27]. Based on the objective of this work, the characteristics of tau protein and its response to exposure to pesticides are highlighted in this review.

Physiologically, the tau protein is involved in myelination processes[16,28], regulation of glucose metabolism[29], rearrangement of microtubules[16], axonal transport[16], iron homeostasis[16], as well as neurogenesis and processes related to learning and memory[16,28]. However, exposure to pesticides may affect the phosphorylation of tau protein and the formation of neurofibrils, resulting in morphological changes in CNS[30,31].

Tau protein is expressed in six different isoforms: 2N4R, 1N4R, 0N4R, 2N3R, 1N3R and 0N3R, depending on their amino and carboxyl groups. These isoforms come from the alternative cutting and splicing of the microtubule associated protein tau (*MAPT*) gene located on position q21 of chromosome 17[16,28]. Tau is a 441-amino acid hydrophilic protein sub-classified into 4 domains. The sites susceptible to

phosphorylation correspond to the amino acids Ser198, Ser199 and Ser202[32]. The amino acids Thr181, Thr205 and Thr217 are associated with the early stages in the development of AD[16,28], while amino acid residues Ser262, Ser396 and Ser404 are associated with formation of aggregates[31].

Tauopathies are pathologies that arise as a result of alterations in the phosphorylation of tau. Often, these alterations are the result of imbalance involving two kinases: Cyclin-dependent kinase 5 (Cdk5) and glycogen synthase kinase 3 beta (GSK-3β), and protein phosphatase-2A (PP2A). The enzymes Cdk5 and GSK-3β are responsible for phosphorylating tau protein, whereas PP2A dephosphorylates the protein. A close relationship has been reported between GSK-3β and PP2A, both of which regulate each other. For example, when GSK-3β is activated, PP2A is inactivated by auto-phosphorylation at its amino acid residue Tyr-307[28,32-34]. Moreover, the regulation of GSK-3β also depends on routes modulated by calcium/calmodulin, and on the ratio guanosine-5'-triphosphate/guanosine diphosphate. Another important alteration in tauopathy is the phosphorylation of proteins associated with microtubules-2 (MAP-2) which are regulated by cyclic adenosine monophosphate. The MAP-2 is important in stabilizing microtubule assembly, and it is intimately bound to tau protein. Thus, it has been reported that multiple axopathies are associated with abnormalities in these pathways[35,36].

In AD, increased hyperphosphorylation of tau forms aggregates in neuronal cytoplasm, resulting in generation of the so-called neurofibrillary tangles and neurotrophic neurites, which are responsible for neurodegeneration[28,31,37,38]. This phenomenon induces morphological changes in dendrites and causes axonal shortening which alters neuronal plasticity and response to neurotransmitters, leading to problems associated with spatial memory, motor skills and learning, all of which are characteristics of AD[33,34,39]. In addition, the release of tau aggregates in the cytoplasm increases immunoreactivity and oxidative stress which influence neuroinflammation[34,40]. Increased oxidative stress over-activates GSK-3β, thereby making tau protein more vulnerable to formation of aggregates and new neurofibrils[41].

Results from multiple studies indicate that exposure to pesticides modifies the mechanisms involved in tau phosphorylation[32-34,42,43] (Figure 1).

Effect of OCs on the tau protein

Table 1 shows 7 studies in which the effect of OCs on tau protein phosphorylation was determined[42,44-49]. Two clinical studies reported that exposure to these pesticides may be associated with polymorphisms in MAPT and microtubule associated protein 1B gene which are related to the formation of tau aggregates[44,45]. Studies have demonstrated that dichlorodiphenyltrichloroethan exposure to an OC altered mitochondrial function, resulting in the formation of tau aggregates, with up-regulations in the expressions of proteins such as synaptosome-associated protein 25 kDa, cytochrome C, enolase A, hemoglobin alpha chain and histone cluster 1, which are characteristic of AD[42,46,47]. Finally, Mir *et al*[48] has shown that exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin induced overexpression of GSK-3β, and hence tau phosphorylation. In all, 4 of the 7 studies described in Table 1 reported increases in tau phosphorylation[42,44,46,48]. Therefore, OCs have been associated with development of tauopathy which leads to axonal instability, mitochondrial dysfunction and neuroinflammation[44].

Effect of OPs on the tau protein

Exposure to OPs (chlorpyrifos, paraquat and malathion) increases the level of hyperphosphorylated tau protein, overstimulates glial cells, and increases the levels tumor necrosis factor-α, interleukin (IL)-6, IL-β, chemokines, NADPH oxidase 2, NADPH oxidase and COX-2, thereby inducing neuroinflammation. Intensified inflammation accelerates pathologies associated with neurodegenerative processes[33,40,50]. Similarly, when OP pesticides are transported through the blood-brain barrier, a process regulated by Na^+ dependent transporters, microglia are activated, resulting in redox imbalance which affects the mitochondrial respiratory chain at mitochondrial complex I, leading to deterioration of CNS function[51,52]. Table 2

provides a breakdown of 18 studies on the effect of OPs on tau protein[31,33,36,37,39,41,49,50,51,53-61]. The only report on cases and controls with OP exposure for more than 2 years showed higher levels of tau phosphorylation in exposed subjects[53]. On the other hand, eight out of eleven studies indicate that exposure to these pesticides increased tau phosphorylation through different mechanisms involving GSK-3β overexpression, increased Cdk5 activity, and decreased expression of PP2A, among other factors (Table 2). Six review studies described in Table 2 reported increases in tau phosphorylation related to greater Cdk5 activity, with changes in regulatory proteins MAPT and MAP-2, and increased oxidative stress, among other changes.

Effect of Cs on the tau protein

There are only a few studies on the effect of C pesticides on tau phosphorylation. It is important to highlight that there are no clinical or epidemiological studies on this topic, to date. Most of the studies analyzed in this review indicate that exposure to Cs led to hyperphosphorylation of tau[32,54,62,63]. Only two studies, reported otherwise[31,64]. Increased hyperphosphorylation may be mediated by increased GSK-3β activity and PP2A inhibition (Table 3). In a murine model, exposure to carbofuran, a C pesticides resulted in neuronal death at the cortex and hippocampus, as well as alterations in spatial memory and learning processes[65]. It is interesting to note that C pesticides are currently being used for their therapeutic potential as AchE inhibitors in different pathologies[64,66,67]. More details associated with the effect of exposure to C pesticides on tau protein are presented in Table 3.

Effect of Ps on the tau protein

Exposure to P pesticides also increases tau protein phosphorylation by modifying the activity of kinase enzymes through over-activating. Contact with P pesticides is associated with increased immunoreactivity that affects cognitive processes, spatial memory, and learning, which are alterations consistent with the development of AD[32,65,68]. Three out the few studies that have been so far published on the effect of Ps

on tau protein, and one review, are shown in Table 4. Amongst the most relevant results reported are increased activity of GSK-3β[34,69], increased neuroinflammation[34,69] and decreased activity of PP2A[34].

Effect of Ns on the tau protein

Studies on the effect of N pesticides on tau protein in humans or experimental models are very few in number. The few reports available highlight the work of Kimura-Kuroda *et al*[70] who found that exposure to 1-100 μM acetamiprid or imidacloprid (both N pesticides) increased intracellular Ca^{2+} influx in cerebellar neurons by activating calcium/calmodulin-dependent kinases, thereby over-stimulating tau protein phosphorylation. In a clinical case report on accidental ingestion of imidacloprid and thiamethoxam, the resultant increase in Ca^{2+} influx altered the kinase response[22]. Another mechanism involved activation of the Wnt pathway, leading to apoptosis[71]. More details are shown in Table 5.

PESTICIDES AND THEIR COGNITIVE IMPLICATIONS

Epidemiological studies have associated pesticide exposure with increased risks of cognitive impairment and AD[72,73]. For example, exposure to OC pesticides has been associated with low scores in the mini-mental test, and with severe cognitive decline[74-77]. Singh *et al*[78] reported that exposure to δ-hexachlorocyclohexane), dieldrin and pp'-dichlorodiphenyldichloroethylene were associated with AD (odds ratio = 2.064, 2.086 and 4.8, respectively; 95% confidence interval). These results are consistent with the cognitive findings[32-34,44,46-48,50,53,54,56,58,60,63,69,79] reported in Table 6, where contact with OCs was associated with increased cognitive impairment, decreased scores in tests evaluating spatial memory, decreased scores in results of tests on evaluation of novelty, and poor performance in tests evaluating attention and problem solving, except for two studies that did not report differences in scores in the tests applied[46,49].

Lin *et al*[80] reported that workers exposed to OPs had cognitive impairment (hazard ratio = 2.21; 95% confidence interval). Hayden *et al*[81] reported that exposure to OPs

increased the risks of MCD and AD (hazard ratios = 1.38 and 1.42, respectively; 95% confidence interval), and Paul *et al*[82] reported that exposure to OPs has been associated with rapid progression to MCD (hazard ratio = 1.94, 95% confidence interval). In the studies analyzed in Table 6, it was found that contact with OPs was associated with decreased MMSE testing and deficiencies in tests involving evaluation of verbal, non-verbal and spatial memory (Table 6). These cognitive findings are related to the results presented by Lin *et al*[80], Hayden *et al*[81] and Paul *et al*[82] referred to above.

Exposure to Cs is also associated with increased risk of developing MCD (odds ratio = 1.98; 95% confidence interval)[80]. Kamboj *et al*[83] showed that exposure of rats to Cs for 28 d performed poorly in Active Avoidance Task, indicating deterioration of cognitive function. Additionally, findings from studies on effect of Cs exposure on cognition have been linked to deterioration in spatial memory and decreased scores in results of tests involving evaluation of novelty (Table 6).

Very few studies have been done on the risk of cognitive impairment and AD due to exposure to P and N pesticides. In Taiwan workers exposed to Ns, approximately 2.9% mortality was reported, which is similar to the value of 3.1% reported for pyrethrins and Ps[22,79]. The results reported in Table 6 indicate that exposure to Ps is related to a deterioration in spatial memory, alteration in problem solving capacity, and increase in cognitive impairment similar to that reported by Estrada Atehortúa *et al*[22]. Besides, according to Phua *et al*[79], exposure to N pesticides resulted in aggravated disorientation, altered mental status, and confusion (Table 6).

Previously, the difficulty in studying the association between exposure to pesticides and prevalence of MCD and AD in humans was attributed to the complexity of obtaining separate data for each pesticide classification, because human exposure usually results from poisoning from multiple pesticides in conjunction with different chemical vehicles[65].

CONCLUSION

Pesticide exposure is associated with increased hyperphosphorylation of the tau protein amino acid residues Ser198, Ser199 and Ser202, Thr205, Ser396 and Ser404. This occurs through the following mechanisms: increased enzyme activity of Cdk5 or GSK-3β, decreased PPA2, mutations associated with the *MAPT* gene, increased neuroinflammatory response, enhanced influx of intracellular Ca^{2+}, and impairment of oxidative phosphorylation. These mechanisms may be related to the pathogenesis of AD. In addition, exposure to pesticides may be involved in lower performance in mini-mental tests, alteration in verbal, non-verbal and spatial memory, decreased response to novelty tests, and reductions in attention and problem-solving potential. One limitation in this study is the small number of publications on Cs, Ps and Ns pesticides and their effects on tau protein. Therefore, there is need to carry out a broader study on these variables in subsequent investigations.

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