

Tracking the footprint of pesticides in Alzheimer's disease

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Abstract

Several studies have indicated that exposure to pesticides is one of the main environmental factors that are associated with neurodegenerative diseases. Indeed, even the chronic exposure to low dosages of pesticides could be associated with some neurological diseases, including Alzheimer's disease (AD). In this review, we have discussed the mechanisms associated between exposure to pesticides and AD. We particularly concentrate on the impacts of pesticides on the oxidative stress, mitochondrial malfunction, formation of A β plaques, and hyper-phosphorylation of Tau proteins, which are important factors in the pathology of Alzheimer's disease.

Introduction

Alzheimer's disease is a brain disorder that gradually influences and decreases several mental abilities in humans. This chronic neurological disease usually has a slow course of progression. One of the most common early symptoms of AD is loss of short-term memory, and the progression symptoms can include speech problems, confusion, mood swings, self-care inability and behavioral issues [1]. However, the exact cause of AD is not known, it seems a multi-factorial process, which may include both genetic and environmental factors, impacting many cellular and molecular processes in the central nervous system (CNS) [2,3].

An exponential increase in the number of people suffering from AD since late 1990s indicates that environmental pollutions may have a significant impact on the incidence of this disease [4,5]. During the past few decades, synthetic insecticides and pesticides, which are toxic for the nervous system, have been extensively used worldwide [6,7]. Beyond affecting pests, these chemicals also affect non-target species and cause air, water and soil pollution [8]. Since most pesticides are fat-soluble and cannot be eliminated from the biological systems easily, they become more concentrated in higher levels of the food chain [9]. Thus, many of the commonly used pesticides remain in the body for a long time [10].

With respect to the organisms that pesticides target, they can be categorized as herbicides, insecticides, fungicides, and raticides [11]. In regards to their active sites, pesticides can be placed in the organochloride, organophosphate and carbamate groups. Chlorinated organic hydrocarbons, such as DDT¹, could divide into dichlorodiphenylethan and cyclodiene compounds. The toxicity level of these molecules varies depending on their degradation persistence and bioaccumulation potential [12].

A number of toxicology studies have shown that some pesticides,

especially insecticides, such as Chlorpyrifos (Organophosphate insecticides), Cypermethrin (type II Pyrethroid insecticides), Maneb (Dithiocarbamate fungicides), and Warfarin (Rodenticides), can cause a considerable damage to the nervous system [13]. Exposure to high doses of insecticides (organophosphates and carbamate) can inhibit the Cholinesterase, which can lead to a continuous production of Acetylcholine, causing symptoms, such as weakness or paralysis [12]. Some organochloride insecticides and pyrethroids have neurotoxic effects on ion channels, which can disturb the balance of sodium/potassium in nerve fibers [14]. The basic function of the organophosphate pesticide is changing the neuronal signal transduction. Different pesticides have different action mechanisms. But, all of them eventually impact the transmission of nerve impulses.

High doses of pesticides can strongly affect the nervous system, but the question is how does exposure to low doses of these toxins affect the body? Many reports have shown that in the areas where people were permanently exposed to pesticides, the prevalence of neurological diseases, such as AD, Parkinson's Disease (PD), Multiple Sclerosis (MS) and polyneuropathy, was notably high [15-18]. Many studies have suggested that there might be a link between exposure to pesticides and the incidence of AD [19-22]. For example, a few epidemiology studies have shown that the prevalence of AD among some farmers who were exposed to organophosphate and organochloride was significantly

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Key words: pesticides, oxidative stress, a β plaque formation, tau hyper-phosphorylation, Alzheimer's disease

Key message: Exposure to pesticides might be a risk factor for Alzheimer's disease

Received: September 05, 2015; **Accepted:** September 25, 2015; **Published:** September 28, 2015

¹Dichlorodiphenyltrichloroethane

higher compared to the rest of populations that had not experienced that exposure [21,23-25]. Singh and his colleagues have shown that use of organochloride pesticides (such as pp DDE², β -HCH³, Dieldrin) and risk of AD are correlated [26], and that β -HCH levels in the serum of AD patients was several times higher compared to the healthy individuals. A study on 180 PD and AD patients who had the genetic risk factors for these diseases showed that individuals who had higher blood levels of the insecticide had a faster progression of AD and PD [27]. Another study reported that the serum levels of DDE⁴ and DDT organochloride derivative compounds were remarkably higher in AD patients compared to the rest of population [28].

Exposure to pesticides at relatively low doses may affect the brain and lead to the loss of nerve cells in certain areas of the brain, which can have severe negative impacts on cognitive function, memory, advertence, mobility function in humans. These changes may eventually lead to AD [21,29].

Possible mechanisms underlying the impacts of pesticides on AD

Oxidative stress and mitochondrial dysfunction

Many human neurological diseases, such as AD, are related to the loss of mitochondrial function. The brain needs a lot of energy to maintain its normal physiological function. In addition, neurons are highly dependent on the energy that is produced in mitochondria [30,31]. Mitochondria are the primary consumers of oxygen and they contain multiple redox enzymes (oxidation/reduction) enabling them to transfer electron to oxygen molecules. Electron acceptor compounds are called oxidizing agents. The molecules that lose their electrons generate reactive oxygen species (ROS) [32]. ROS include reactive molecules and free radicals derived from molecules, which have oxygen atoms in their structures and have a short half-life, such as superoxide ($O_2^{\cdot-}$), hydroxyl (OH^{\cdot}), and hydrogen peroxide (H_2O_2) [33] (Figure 1). Furthermore, mitochondria play important roles in apoptosis. During the early stages of apoptosis, the potential difference

in mitochondrial membrane is lost and some mitochondrial proteins, such as cytochrome c, are released into the cytosol [34].

Mitochondria are the main sites of ROS synthesis and have many antioxidant defense systems that can dispose the produced ROS. When mitochondria are damaged, the antioxidant system is compromised, resulting in an increased production of ROS and higher levels of oxidative stress, which leads to further mitochondrial damage. Imbalanced levels of cellular ROS leads to cellular damage and cell death [35,36]. ROS can be indirectly evaluated by measuring the levels of antioxidants and antioxidant enzymes. Some biochemical and cellular studies performed on postmortem brains of AD and AD transgenic mice have suggested that oxidative stress, mitochondrial damage and synaptic dysfunction are primary cellular changes in the development and progression of AD [37]. In fact, high levels of ROS have been reported to be correlated with the pathology of AD [38,39].

At the present time, most of the pesticides that are being used in many countries work based on toxic effects of oxidative stress, leading to an excessive production of reactive oxygen and nitrogen species (ROS, RNS), which can influence the level of antioxidants and inhibit the function of many cellular enzymes [40,41]. Some fungicides (e.g. Maneb) and insecticides (e.g. Rotenone) disturb neural functions through the suppression of mitochondrial function, which leads to an increased free radical production. Some herbicides also cause an uncoupled oxidative phosphorylation, which increases the levels of free radicals [42,43].

Exposure to pesticides, such as Zineb and Glyphosate, enhances the levels of oxidative stress in the brain and liver, which is correlated with the loss of cardiolipin and transmembrane potential content and simultaneously increases the peroxidation of fatty acids, especially in the substantia nigra [44,45]. To date, many studies have shown an increase in lipid peroxidation in AD [46-50] during which lipids are attacked by ROS, and chain reactions with free radicals lead to the production of lipid peroxidation products.

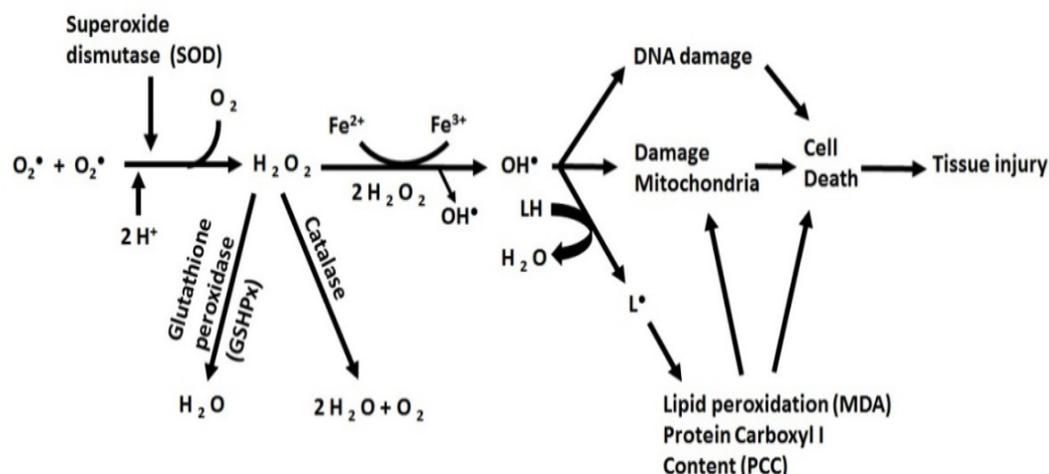


Figure 1. Effects of oxidative stress on cells: Lack of or excessive ROS production can interfere with the electron transfer change in mitochondria, which is a known risk factor in the pathology of Alzheimer's disease.

²p,p'-Dichlordiphenyldichlorethen

³ β -Hexachlorocyclohexane

The impact of increased oxidative stress in aging and neurological degeneration is complicated. Oxidative stress is a result of imbalanced production and disposal (detoxification) of reactive oxygen species (ROS). ROS products are inevitable molecules that have a double-edged sword effect on biological systems. The balanced levels of ROS play crucial roles in maintaining many vital activities in cells, especially neurons. On the other hand, very high levels of ROS can lead to oxidation of important biomolecules, such as DNA, RNA, proteins and lipids, which can negatively affect cellular function and survival [51]. Several reports have shown that oxidative damages to DNA and RNA can cause rupture of double-stranded DNA, protein crosslinking, organic base alterations. Furthermore, a large number of DNA fractures have been found in the hippocampus and cortex of AD patients [52,53].

In addition to increased oxidative products in AD patient, serum levels of antioxidants, such as albumin, bilirubin, uric acid, lycopene, vitamin A, vitamin C and vitamin E are significantly reduced [54,55], and the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and oxygenase in different brain regions of AD patients, including frontal and temporal cortex, are highly reduced [45,56,57].

Beta amyloid plaque formation

Many studies have indicated that the amyloid protein derived from *amyloid precursor protein* (APP) is the main factor in the pathogenesis of AD. APP is a transmembrane protein with unknown functions in different cell types, such as neurons [58]. This protein is attached to APP-like proteins, such as APL1 and APL2 [59]. When APP is proteolyzed by the enzymes β - or γ - secretase, it can lead to

the production of 38-43-amino acid peptides. $A\beta$ molecules can form soluble, flexible and polymorphic oligomers. $A\beta_{40}$ and $A\beta_{42}$ isomers are the most common proteins [60]. But, accumulation of $A\beta_{42}$ fibrils is more common in AD [61]. In the neurons of healthy individuals, the amounts of $A\beta$ fragments are low and they can be easily lysed. But, overproduced $A\beta$ fragments cannot be cleared in neurons, which can form spherical structures that are believed to play important roles in the pathogenesis of AD. $A\beta$ plaques may have an impact on several age-related diseases by enhancing oxidative stress as well as increasing $A\beta$ monomers or small oligomers, which can cause neuronal damage [62]. A specific isoform of apolipoproteins E (APOE4) is one of the important genetic risk factors in AD. The pathological interactions between this protein and APP has been shown to be correlated with AD [63]. Moreover, some mutation in the *triggering receptor expressed on myeloid cells 2* (*TREM2*) gene are a risk factor for developing AD. The normal interaction between the TREM2 protein and microglia in the brain is necessary for the activation of these cells to clean up plaques. When this gene is mutated, it can interfere with its normal effect on the mentioned microglia's cleaning function, increasing the chance of developing AD [64]. $A\beta$ is produced in the perikarya of neurons and transmitted to the axons terminal. Recent studies have shown that $A\beta$ can accumulate in the mitochondrial membrane and induce the production of free radicals. This phenomenon can reduce the activity of mitochondrial complex IV. As a result, the production of ATP is reduced, resulting in mitochondrial dysfunction and neuronal death [65]. Many studies have suggested that neuronal damage, synapsis failure and neurofibrillary network (NFT) are pathological symptoms of AD [66,67] (Figure 2).

Long term exposure to some organophosphate pesticides, such as

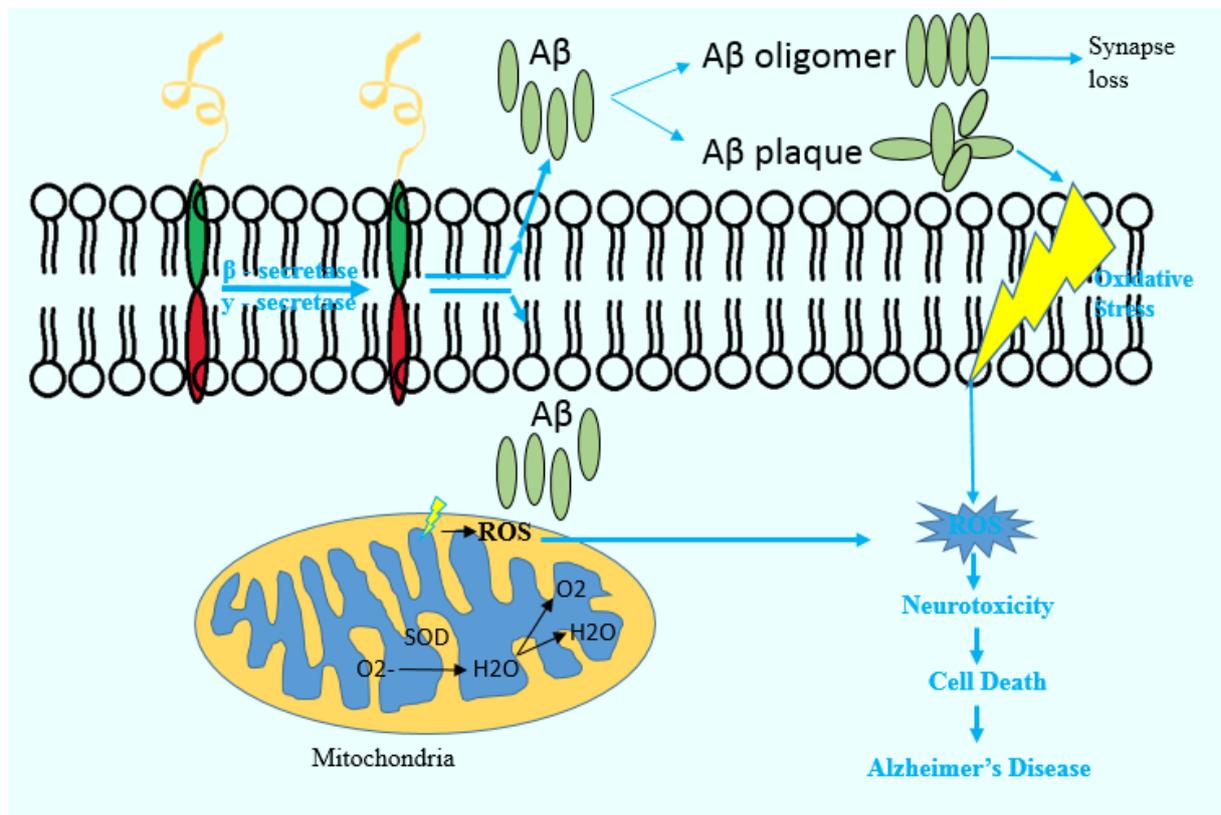


Figure 2. $A\beta$ plaques and oxidative stress. β - and γ - secretase cleave APPs and produce $A\beta$ units. Outside cells, these proteins can lead to synapse loss and oxidative stress. Inside cells, $A\beta$ units can increase the generation of ROS, causing neurotoxicity and cell death.

Chlorpyrifos, increase beta-amyloid levels in the brain [68]. Organic chlorinated pesticides are fat-soluble toxins that remain stable in the environment and food chains. In humans, hemostatic factors are stored between organochloride toxins in adipose tissues and their concentration in the blood circulation is not clear [69]. Although these toxins are consistently stored in fat tissues, they can be released in the blood after a rapid weight loss [70]. According to Jason Richardson, long-term exposure to toxins, such as DDT and DDE, can increase the levels of APP, which is correlated to the formation of A β plaques. Moreover, in AD patients who often lose weight, the release of such organochlorinated factors in the blood accelerates the progression of AD [28].

Tau Phosphorylation

Biochemical and cellular studies on rats continuously exposed to pesticides, such as Carbofuran and deltamethrin, have shown an excessive tau phosphorylation occurring by the activation of glycogen synthase kinase-3 β and inhibition of protein phosphatase-2A [71]. Tau phosphorylation leads to the formation of the two stranded and intertwined neurofibrils in the nerve cells [72], which can lead to the disintegration of microtubules, loss of cellular structure and malfunction in the neural functions [73]. At first, it may disrupt the biochemical communication between neurons followed by cell death [74] (Chun W 2007). A β aggregation and phosphorylated tau can block cell organelle transfer, including mitochondria, to synaptic terminals where high ATP is required. For instance, in a study using neuronal and non-neuronal cells with multiple Tau mutants, this protein was able to block the backward movements of vesicles and reduce cell organelle transportation by blocking microtubules. Tau was also involved in reducing mitochondrial ATP in dendrites and synapses [75]. In addition, another study on the effect of Tau on vesicle and organelle transportation in primary cortical neurons, retinal ganglion cells and neuroblastoma showed that Tau inhibited the kinesin-dependent transmission of peroxisomes, neurofilament (NF) and derivative vesicles in these cells. The absence of peroxisomes makes the cells vulnerable to oxidative stress and cause neuronal damage. This study also indicated that Tau blocks APP transfer to axons and dendrites and interferes with the axonal transport [76].

Conclusion

The use of pesticides for pest control has become common around the world. Pesticides are found in the air we breathe, the water we drink and the food we eat. Thus, pesticides are associated with a wide spectrum of health problems, including neurological diseases, especially AD. Exposure to low doses of pesticides for a long time can enhance the formation of A β plaques, resulting in the increased level of oxidative stress. ROS produced in cytoplasm reduce the expression levels of antioxidants, leading to cell death. ROS produced in the mitochondria and intracellular tau filaments are able to create neuronal toxicity. Eventually, neural death occurs. All these pathologic phenomena can lead to or accelerate AD. Further research is clearly required to shed light on the mechanisms in which pesticides affect the cellular risk factors of AD. The present review underlines the necessity of designing pesticides with zero side effects on the human body, especially the nervous system, as well as developing other methods of dealing with pests, such as using biological pest control and insect breeding interference- based genetic engineering practices.

Acknowledgments

We thank Amanda Marie Di Battista from Dr. G. William Rebeck's lab at Georgetown University for her constructive comments on our manuscript.

Conflict of interest

The authors reveal no conflict of interest.

Author contribution

EG, MT, PP, SF wrote the manuscript. NGA and MT edited the manuscript.

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