Review

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Dichlorodiphenyltrichloroethane (DDT) induced extracellular vesicle formation: a potential role in organochlorine increased risk of Parkinson's disease

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A number of studies have demonstrated that rural living and exposure to pesticides such as dichlorodiphenyltrichloroethane (DDT) highly increase the chances of developing Parkinson's disease. In a previous work, we have found that DDT leads to the formation of vesicular buds that are released from the cells upon fusion of an intermediate endocytic compartment with the plasma membrane. Since extracellular vesicles like exosomes have been implicated in the development of neurodegenerative diseases through the propagation of neurotoxic misfolded proteins from neuron to neuron, in this minireview we propose that organochlorine pesticides could enhance the risk of neurodegenerative diseases by increasing the formation of exosomes.

Key words: dichlorodiphenyltrichloroethane, Parkinson's disease, exosome, organochlorine

INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disease that affects primarily dopamine neurons in the substantia nigra, whose main treatment consists in drugs that increase dopamine receptor stimulation (Aloisi et al. 2011, Connolly and Lang 2014). A number of studies have demonstrated that rural living and exposure to certain pesticides, such as dichlorodiphenyltrichloroethane (DDT), highly increase the chances of developing Parkinson's disease (Van der Mark et al. 2012, Saeedi Saravi and Dehpour 2016).

DDT is an organochlorine known for its pesticide properties and for its negative effects on human health. This compound, by preventing the activation of the voltage gated sodium channels, causes an uncontrolled neuronal firing that is known to provoke important muscle spasms which in turns lead to death of insects (Dong 2007). It was widely and abundantly used between the 1940s and the 1970s and finally banned in most countries of the world for its toxicity to the endocrine system (Mnif et al. 2011). Nevertheless, DDT is still routinely used in some developing country, most of them in Africa, to fight mosquitoes that carry malaria (Channa et al. 2012). Furthermore, due to its extremely long half-life (up to 30 years), DDT is linked to several health and social problems which are due to its accumulation in the environment and its biomagnification properties in living organisms (Mansouri et al. 2017). In this review, we will summarize data consistent with DDT induced vesicle formation and we will discuss how this phenomenon could explain the increased risk of Parkinson's disease in patients previously exposed to this pesticide and other organochlorine derivatives.

A new property of DDT on plasmamembrane

During a screening of several environmental factors that alter thyroid homeostasis, Santini and others (2003) found that organochlorine derivative like DDT and Aroclor disrupts thyroid stimulating hormone (TSH) receptor function. We studied in detail the mechanism by which DDT causes this effect and we demonstrated that DDT inhibits TSH receptor function by modifying the lipid organization of the cell membrane (De Gregorio et al. 2011, Picchietti et al. 2009, Rossi et al. 2007). The fact that DDT is highly lipophilic might count for these inhibitory effects on the TSH receptor activity, inasmuch highly lipophilic molecules are known to deplete the cholesterol content in the cell membrane, which results in a defective organization of

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the raft microdomains that contain the TSH receptors (De Gregorio et al. 2011). In particular, DDT caused receptors and lipid rafts to become highly separated and dislodged along opposite cell poles, and by this mechanism it inhibits the TSH receptor whose signalling depends on its constitutive association with lipid raft microdomains (Latif et al. 2003).

Unexpectedly, DDT also led to the formation of vesicular buds that were released from cells upon fusion of an intermediate endocytic compartment with the plasma membrane (De Gregorio et al. 2011). It is plausible that DDT induces membrane shedding *via* specific interaction with raft microdomains of the plasma membrane. Membrane shedding and vesicle formation are not necessarily related with the high DDT mediated lipophilicity. Experiments with diphenylethylene, a structurally related compound with no chlorine atoms, demonstrated that the TSH receptor function was not affected and that neither raft microdomains nor vesicles were altered (De Gregorio et al. 2011, Rossi et al. 2009). The results observed with DDT then, suggested that the diffuse chlorination of DDT, together with its high lipophilicity, were responsible for the effects on the vesicle formation and on TSH receptor activity, and more importantly that, other organochlorine pesticides might show similar properties. For instance, Aroclor 1254, an organochlorine derivative structurally related to DDT, clearly alter the function of TSH receptors (Rossi et al. 2007). Furthermore, Choi and others (2012) have shown that several polychlorinated biphenyl modify the blood brain barrier permeability in mice by altering lipid rafts organization, and this is consistent with the data of Eum and colleagues (2015) who demonstrated that the polychlorinated biphenyls PCB153 disrupts the brain endothelial barrier by altering the function of lipid raft-associated proteins such as phosphatase 2A and matrix metalloproteinase-2.

The observation that lipid rafts are present in vesicular membranes and participate in vesicle formation (de Gassart et al. 2003, Tan et al. 2013), makes it likely that organochlorine derivatives might increase vesicle formation by simply modifying the raft distribution in the cell plasma membrane.

To our knowledge, there is no other published evidence that organochlorine derivatives like DDT induce the formation of extracellular vesicular bodies in mammalian cells. Though, this is not surprising because previously these vesicles were thought to be cellular artifacts and their formation was neglected. Nevertheless, there is evidence that DDT induces reorganization of membranes. For instance, in 1985 Osborne reported, as an unpublished result in a book chapter, that in the stick insect neurohaemal organ (a neural organ that directly release hormones in the blood stream) 1 μM DDT induces a pronounced increase in the number of exocytotic profiles in nerve terminals following a 20 minute incubation. Remarkable, this effect of DDT was observed in the absence of connection of the nerve terminals with the rest of the nervous system, suggesting that DDT was acting locally on the neuronal membrane. Furthermore, formation of multivesicular bodies has been reported in neurons when they were exposed to toxic level of DDT (Von Bartheld and Altick 2011). These data suggest that DDT tends to remodel the ultrastructure of the membranes with the formation of vesicles.

At the moment, it is not clear from our data whether the vesicles formed in CHO cells after DDT can be considered exosomes or microvesicles. Exosomes are extracellular vesicles that are released from cells upon fusion of an intermediate endocytic compartment, the multivesicular body, with the plasma membrane and have size of 40–100 nm. Microvesicles are shed from the plasma membrane and have a larger size of 100–1000 nm (Raposo and Stoorvogel 2013). Circulating vesicles are composed of both exosomes and microvesicles, and current purification methods, do not allow one to fully discriminate between them. Furthermore, both of these vesicles have been shown to play a role in cell-to-cell communications (Raposo and Stoorvogel 2013), even though the majority of studies refer to exosomes.

Exosomes, a bubble ride for synuclein

At the time we discovered the induction of vesicle formation by DDT, the field of exosomes was just at its beginning (Lopez-Verrilli and Court 2013), and therefore, these findings were simply described rather than thoroughly analysed for their potential toxicological effects. In recent years, a plethora of data have demonstrated the crucial role played by exosoms in mediating physiological and pathophysiological cell-to-cell communications. For instance, exosomes have been implicated in the development of neurodegenerative diseases through the propagation of neurotoxic misfolded proteins from neuron to neuron (Properzi et al. 2015), and in the propagation of diseases-associated with misfolded prion proteins (Cervenakova et al. 2016). As a matter of fact, α-synuclein, a soluble monomeric protein, whose misfolding is closely associated with the development of Parkinson's disease, seems to be transferred from neuron to neuron by exosomes. In particular, it has been shown that toxic α-synuclein oligomers are released extracellularly in a exosome-mediated manner when the autophagic mechanism destined to degrade them fails to accomplish its goal (Danzer et al. 2012). In other words, the secretion of misfolded α-synuclein oligomers is strongly influenced by autophagic activity, for if autophagy fails in sick neurons, then α-synuclein would be released through exosomes and incorporated into healthy neurons causing the disease to spread.

Furthermore, in experiments with isolated exosomes from neuroblastoma cells, Grey and others (2015) found that exosomes catalyze the aggregation process of α-synuclein in a manner similar to that performed by α-synuclein fibrils at low concentration. In particular, it has been demonstrated that the exosomes abbreviate the time of aggregation for this protein, which suggests that they provide the catalytic environments for α-synuclein nucleation. Interestingly, vesicles isolated from extracted exosome lipids were indeed able to accelerate this aggregation process, suggesting that the lipids in exosomes were sufficient for the observed catalytic effect (Grey et al. 2015). In particular, the acceleration of α-synuclein aggregation as observed in the presence of exosomes was reproduced by vesicle preparations composed of fluid lipid bilayers that contain gangliosides, a type of lipid that is highly enriched in rafts (Pike 2003).

Potential mechanism of neurotoxicity by DDT induced extracellular vesicle formation

Due to its high lipophilcity, DDT can easily cross the blood-brain barrier and accumulate in the brain, where it may persist mostly as the DDT residue dichlorodiphenyldichloroethylene (DDE), detectable in patients exposed to the pesticide (Fleming et al. 1994). Therefore, we propose that exposure to DDT could increases the risk of developing Parkinson's disease, by inducing the formation of microvesicles/exosomes and consequently by promoting the oligomerization and spreading of α-synuclein oligomers among neurons.

The toxicological relevance of our findings is based on the concentration at which DDT exerts its effect that should be comparable with the one detectable in the environment. The literature available for DDT, due to its use as an insecticide worldwide, indicates around 10 μM the serum concentration of this compound in chronically exposed subjects (Chen et al. 2005, De Jager et al. 2006), and this is the concentration at which DDT begins to have an effect in our experimental setting (Rossi et al. 2007).

As mentioned above, this hypothesis could also be extended to other organochlorine pesticides. For instance, Chhillar and colleagues (2013) measured the blood-concentrations of several organochlorine pesticides in 70 Parkinson patients and 75 controls. Strikingly, they found that the most frequently detected organochlorine was dieldrin and that it was present in 9.3% of the controls and, amazingly, in 61.4% of the patients with the Parkinson's disease. β-Hexachlorocyclohexane (β-HCH) was also detected and was the stronger predictor of the disease, with an odds ratio of 2.566, meaning that for every additional unit of β-HCH, patients had 2.566 more chances of being fatally affected by the Parkinson's disease (Chhillar et al. 2013). Exosomes have also strongly been associated with the spreading of toxic amyloid β and hyperphosphorylated tau (the activation of the apoptotic signal) among cells, and therefore contributing to the neuronal loss that characterizes the Alzheimer's disease (Malm et al. 2016). Furthermore, as mentioned above for α-synuclein, an accelerated aggregation of amyloid β, as caused by the gangliosides of the exosome membrane, has been observed in people affected by the Alzheimer's disease (Okada et al. 2008). In a recent study, Singh and others (2013) found significantly high levels of organochlorine pesticides, β-HCH, dieldrin, and DDE, in patients with Alzheimer's disease compared to controls. An additional case study by Richardson and colleagues (2014), found that people suffering from, Alzheimer's disease had DDE levels in their bloodstream four times higher than controls which is consistent with the concept that past exposure to pesticides can increase the risk of developing Alzheimer's disease. These studies identify for the first time a strong environmental risk factor for Alzheimer's disease.

CONCLUSIONS

In conclusion, in this minireview we propose a mechanism by which organochlorine pesticides could increase neurodegenerative diseases. Their high lipophilicity, in fact, would allow these pesticides to enter and accumulate in the brain. There they would alter the organization of neuronal membranes and increase the formation of microvesicles/exosomes, that would eventually catalyze the aggregation of proteins, as mentioned above (Grey et al. 2015). These vesicles would then spread the misfolded protein oligomers to other neurons, thus promoting neuronal degeneration. Therefore, while any increase in membrane shedding and vesicle formation, as caused by DDT exposure, could be intended as a cell attempt to get rid of contaminated lipid rafts, the mechanism could turn out to have a negative impact on heath. A cartoon describing how DDT could change membrane properties and by this secretion of vesicles is presented in Fig. 1.

At the moment this is only a speculative hypothesis that needs to be rigorously tested. For instance, we have to prove that vesicle formation occurs in dopamine neurons treated with DDT. For this, primary cultures of midbrain dopaminergic neurons could be a suitable means to test this hypothesis. Furthermore, if vesicle formation occurs in these neurons, we have to prove that they are functional cargo-containing vesicles able to transfer particles between cells, and in particular that these vesicles contain α-synuclein aggregates.

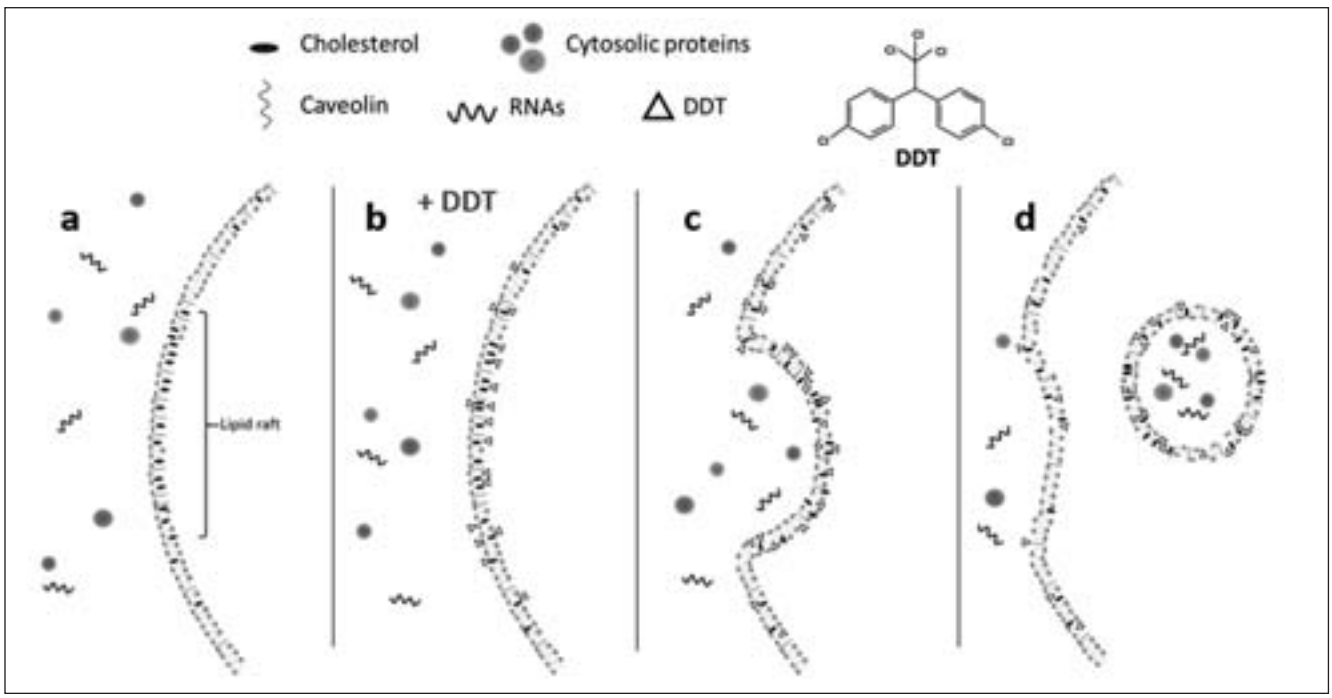

Fig. 1. Formation of vesicular buds and shedding in presence of DDT. A lipid raft (A) is a specialized membrane domain enriched in certain lipids, like cholesterol, and proteins. Do to its high lipophilicity, and probably to its diffuse chlorination, DDT accumulates in cholesterol rich regions like lipid rafts (B). The membrane try to get rid of contaminated lipid raft with the formation of a vesicular bud (C) and shedding of the vesicle (D).

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