Paraoxon: An Anticholinesterase That Triggers an Excitotoxic Cascade of Oxidative Stress, Adhesion Responses, and Synaptic Compromise

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Abstract

The anticholinesterase paraoxon (Pxn) is an organophosphate (OP) and the active metabolite of the insecticide parathion. It potently inhibits the enzyme acetylcholinesterase and leads to enhanced glutamate release, diminished GABA uptake, oxidative damage, and neurodegeneration. The resulting increased levels of acetylcholine can trigger seizures and cause neuronal and excitotoxic damage in the brain. The brain susceptibility related to anticholinesterase toxins extends beyond potential brain damage and death from toxic levels of the agent. Asymptomatic low-level exposure to such toxins can also leave the brain vulnerable or even cause it to exhibit neurological problems later in life. The actions of Pxn and similar neurotoxins have been studied in order to examine the events associated with anticholinesterase toxicity in the brain. A recent study demonstrated that Pxn exposure initiates a pathogenic cascade involving seizure events and subsequent signs of damage including unique presynaptic vulnerability and associated behavioral deficits. In addition, Pxn-mediated synaptotoxicity is also associated with enhanced production of oxidative stress as well as integrin adhesion responses. These findings provide a better understanding of the molecular events involved in Pxn toxicity.

Keywords: Paraoxon, neurotoxicity, excitotoxicity, anticholinesterase, synapse decline

Paraoxon (Pxn), an anticholinesterase toxin, is in the organophosphate (OP) class of compounds that includes insecticides and military nerve agents (e.g. soman and sarin). Exposure to OP toxins is one of the most common causes of poisoning worldwide, affecting nearly 3 million people each year, of which approximately 15% die as a result of the poisoning (Eddleston et al.,

2008). Exposure can occur through drinking contaminated water, breathing vapors of the toxins, or subjecting a person's skin to contact with the toxin. In the central nervous system (CNS), such agents classified as neurotoxins and environmental toxins alter cholinergic, glutamatergic, and GABAergic pathways and can lead to seizures, brain damage, and different neurological syndromes.

Understanding the toxic action of anticholinesterase compounds is vital in order to identify pathogenic steps involved and which of these steps lead to disruptions in synaptic integrity and communication. Pxn, the oxidized active metabolite of the insecticide parathion, has become a common research target to study anticholinesterases (Krutak-Krol and Domino, 1985; Harrison et al., 2004; Todorovic et al., 2012; Deshpande et al., 2014). Pxn causes the accumulation of acetylcholine in synapses and results in a cholinergic crisis in the brain (Wei et al. 2014). Enhanced levels of acetylcholine can trigger seizures, long-term behavioral changes, elongated epileptiform action, and reduced cognition (Sánchez-Santed et al., 2004; Millett, 2006; Prager et al., 2015). In humans, acute OP poisoning also leads to respiratory failure and, hence, can cause death (Eddleston et al., 2005).

The brain's susceptibility related to anticholinesterase toxins extends beyond brain damage and death from toxic levels of the agent. Asymptomatic low-level exposure can also leave the brain vulnerable to subsequent brain insults (see Munirathinam and Bahr, 2004). Even neonatal low-doses of exposure to toxic substances such as pesticides can potentiate brain vulnerability to different types of insults in adulthood (Eriksson and Talts, 2000). In addition, repeated exposure to related pesticides has been linked to an increased risk for Alzheimer's disease later in life (Hayden et al., 2010; Sánchez-Santed et al. 2016). Nerve agent exposure is particularly detrimental to the neurodevelopmental processes underlying synaptic connectivity and cognitive ability (Rotenberg and Newmark, 2003). As a result, children are particularly vulnerable to anticholinesterase toxin exposure.

Animal models have been used extensively to understand the effects of anticholinesterase insults and their relationship to brain damage in humans. Harrison and colleagues (2004) found that, in guinea pigs, Pxn caused seizures similar to those caused by the nerve gas soman in humans. Likewise, the anticholinesterase potency of Pxn was similar in macaques and humans (Worek et al., 2011). Furthermore, Rosenberg and colleagues (2017) showed that anticholinesterase-exposed macaques presented severe signs of toxicity (e.g. fasciculations, miosis, salivation, and convulsions), and many died in less than seven hours post-application. This type of research potentially offers an approach to evaluate and better understand the underlying mechanisms of toxins with actions that trigger brain disturbances.

Certain neurotoxins like Pxn act not only on the cholinergic network, but also affect other neurotransmitter systems involved in excitotoxic propagation. Pxn enhances glutamatergic transmission on hippocampal granule cells. This effect is postulated to occur principally through presynaptic mechanisms (Kozhemyakin et al., 2010). It is noteworthy that the stimulation of nicotinic receptors by itself leads to an increase in glutamate release and thereby increases synaptic transmission in the hippocampus (Sharma and Vijayaraghavan 2003; Alkondon and Albuquerque 2004). Similarly, anticholinesterase agents such as Pxn lead to an over-stimulation of nicotinic and muscarinic receptors through the accumulation of acetylcholine. Pavlovsky and colleagues (2003) previously reported the interaction between these two systems and showed acetylcholine-dependent enhancement of glutamatergic excitatory transmission. Subsequently, Mohammadi and colleagues (2008), also found GABA uptake was significantly reduced in both the cerebral cortex and hippocampus of Pxn-treated rats. Such evidence suggests that acute exposure to anticholinesterase agents not only triggers a cholinergic crisis but occurs in correspondence with the release of excitotoxic levels of glutamate from excitotoxic-sensitive regions of the brain.

Excitotoxic insults are thought to be involved in dendritic and synaptic damage, early toxicological signs that lead to neuronal dysfunction and memory impairment (Munirathinam and Bahr, 2004; Raveh et al., 2002, 2003). Functional imaging during an excitotoxicity study suggests that during seizures, limbic structures such as the amygdala, piriform cortex, entorhinal cortex, and hippocampus are activated (Clifford et al., 1987). Interestingly, hippocampal neurons exhibit an enhanced vulnerability to different types of neuropathogenesis (Mattson, 1990; Bahr et al., 1994), and organophosphate compounds have a significant effect on this brain region (Crino et al., 2002 ; Harrison et al., 2004), particularly due to the high density of cholinergic and glutamatergic innervations.

Brain damage caused by organophosphate intoxication is not restricted to the primary event induced by the poison, but secondary events associated with the anticholinesterase-induced cellular toxicity were observed across several studies in different cell types and brain regions (see Table 1). In the hippocampus, the anticholinesterase effect was observed *in vitro* and *in vivo* by Nallapanemi and colleagues (2006), confirming the ability of Pxn to inhibit the enzyme acetylcholinesterase in this brain region. Pxn exposure comprises different cellular disturbances such as reactive astrocytes, synaptic marker changes, morphological changes, and oxidative stress (Kang et al., 2011; Yu et al., 2012). As found by Jafari and colleagues (2012), Pxn-mediated oxidative damage presented itself not only in brain tissue but also in other organs such as the liver, heart, kidney and spleen.

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Table 1.
Pxn-induced cellular disturbances in different brain regions

Molecular Changes	Brain Region / Cells	Reference
reduced cholinesterase activity	hippocampus	Nallapanemi et al., 2006
	neuroblastoma cell line	Ehrich et al., 1997
enhanced glutamate release	hippocampus and amygdala	Kozhemyakin et al., 2010
diminished GABA uptake	hippocampus and cortex	Mohammadi et al., 2008
reduced cell viability	neuroblastoma cell line	Prins et al., 2010
oxidative damage	human salivary gland cell line	Prins et al., 2014

In a recent study of Pxn exposure using stable explants of brain tissue, indications of oxidative stress and induced alterations to β1-class integrin adhesion molecules were found in correspondence with synaptic compromise (Farizatto et al., 2017). This synaptic compromise was characterized by selectivity of the presynaptic components it affected. Note that, as shown in Table 2, the new study found Pxn-mediated synaptotoxicity associated with oxidative stress as well as integrin adhesion responses both *in vitro* and *in vivo*. These molecular indicators of toxicity show consistency with regards Pxn-mediated cellular changes across both models. These results further indicate the usefulness of screening studies with brain slices to better understand this type of toxin exposure so that it may be properly treated in such a way that offsets damage caused by the toxic cascade.

Table 2.
Pxn exposure leads to oxidative, adhesive, and synaptic changes in both *in vitro* and *in vivo* experiments.

Toxic Changes	Pxn-treated hippocampal slice cultures	Pxn-treated rats
Oxidative stress	increased 4-HNE adducts	increased 4-HNE adducts
Adhesion response	enhanced β1 integrin levels	enhanced β1 integrin levels
Synaptic decline	reduced synapsin IIb and synaptophysin levels	reduced synapsin IIb levels

OP agents like Pxn may initiate synaptic deterioration in which the presynaptic markers appear to be more susceptible than postsynaptic markers (Kozhemyakin et al., 2010; Farizatto et al., 2017). The synaptic decline profile induced by Pxn can be compared to 1) synaptic disruption reported in excitotoxicity studies using hippocampal slice cultures (Bahr et al., 2002; Karanian et al., 2005; Piwońska et al., 2016) and 2) hippocampal slice synaptic declines from protein accumulation stress studies (Bendiske and Bahr, 2003; Butler et al., 2007; Wisniewski et al., 2011). Synaptic pathology after Pxn exposure has been shown *in vitro* and *in vivo* (see Table 2). The illustrative image in Figure 1 shows the synaptic events that are linked to inhibition of acetylcholinesterase. Pxn exposure leads to excess of acetylcholine activity and enhanced glutamate release, and one or both of these changes can lead to seizure, excitotoxicity and brain damage. These findings suggest that Pxn affects the native composition of important CNS synapses and their control of important brain functions.
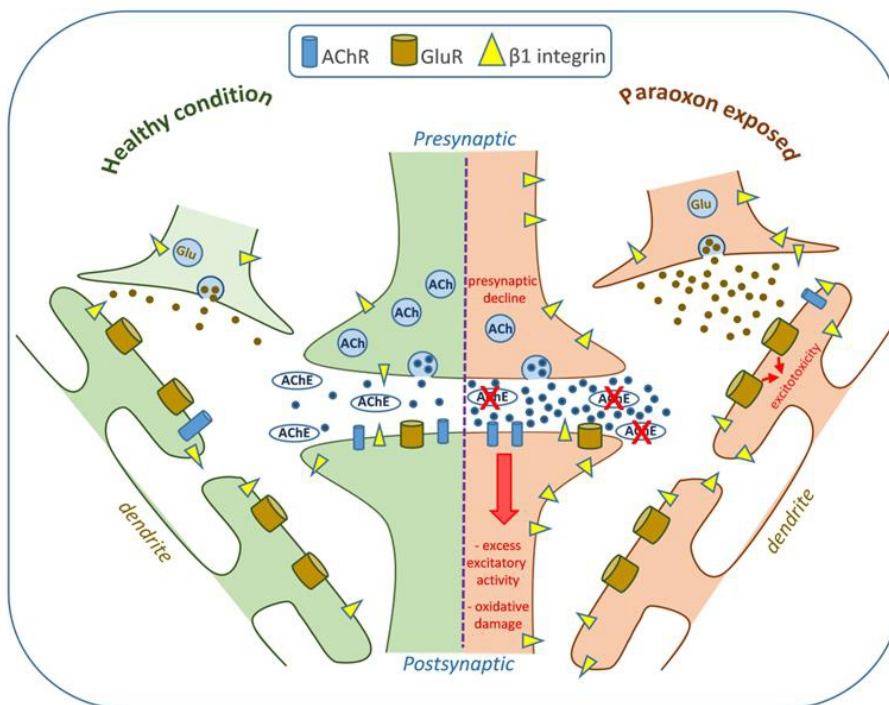
Figure 1: Schematic Illustration of paraoxon-mediated cellular disturbances on brain tissue.

The anticholinesterase paraoxon leads to over-excitatory activity through excess of acetylcholine and enhanced glutamate release in the synaptic cleft, which elicits a cholinergic crisis and excitotoxic damage. Note, the potent inhibition of the enzyme acetylcholinesterase (AChE) by paraoxon exposure also causes presynaptic compromise, adhesion response and oxidative damage.

The degradation of the neurotransmitter acetylcholine by acetylcholinesterase is critical for the maintenance and homeostasis of synaptic integrity and communication. Acute and chronic exposure to compounds like Pxn can lead to neurotoxic effects associated with the human conditions called cholinergic syndrome, intermediate syndrome, organophosphate-induced delayed polyneuropathy, and chronic organophosphate-induced neuropsychiatric disorder. It is very important to understand the actions of related neurotoxins and environmental toxins on synaptic mechanisms and CNS pathways, including cholinergic, glutamatergic, GABAergic, as well as antioxidant systems. The brain's susceptibility to anticholinesterase toxins and the distinct effects on synapses can leave exposed individuals vulnerable to symptoms and neurological problems for the rest of their lives.

Acknowledgements

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