



Published in final edited form as:

Neurobiol Dis. 2020 January ; 133: 104457. doi:10.1016/j.nbd.2019.04.013.

Antioxidant drug therapy as a neuroprotective countermeasure of nerve agent toxicity

Jennifer N. Pearson-Smith, Manisha Patel*

Department of Pharmaceutical Sciences, University of Colorado, Anschutz Medical Campus, 80045, United States of America

Abstract

The use of chemical warfare agents is an ongoing, significant threat to both civilians and military personnel worldwide. Nerve agents are by far the most formidable toxicants in terms of their lethality and toxicity. Nerve agents initiate neurotoxicity by the irreversible inhibition of acetylcholinesterase and resultant accumulation of acetylcholine in excitable tissues. *The cholinergic toxidrome presents as miosis, lacrimation, diarrhea*, fasciculations, seizures, respiratory arrest and coma. Current medical countermeasures can attenuate acute mortality and confer limited protection against secondary neuronal injury when given rapidly after exposure. However, there is an urgent need for the development of novel, add-on neuroprotective therapies to prevent mortality and long-term toxicity of nerve agents. Increasing evidence suggests that pathways other than direct acetylcholinesterase inhibition contribute to neurotoxicity and secondary neuronal injury. Among these, oxidative stress is emerging as a key therapeutic target for nerve agent toxicity. In this review, we discuss the rationale for targeting oxidative stress in nerve agent toxicity and highlight research investigating antioxidant therapy as a neuroprotective medical countermeasure to attenuate oxidative stress, neuroinflammation and neurodegeneration.

Keywords

Oxidative stress; Organophosphates; Acetylcholinesterase; Reactive oxygen species

1. Introduction

Preparedness against chemical warfare threats represents a critical, yet unmet need of current research efforts. To this end, in 2006 the National Institutes of Health launched the Countermeasures Against Chemical Threats (CounterACT) Program to encourage the identification of mechanisms of toxicity and the development of medical countermeasures to mitigate consequences of such exposures (Jett and Yeung, 2010). Chemical warfare agents are highly toxic compounds, exposure to which has the potential to result in mass casualties.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

*Corresponding author at : 12850 E. Montview Blvd V20-C238, Aurora, CO 80045, United States of America, Manisha.Patel@ucdenver.edu (M. Patel).

Conflict of interest statement

Dr. Patel served as a consultant for Aeolus Pharma which developed catalytic antioxidants for human use.

Exposure to such chemicals can be accidental or intentional, as was highlighted recently by the 2013 sarin gas attack on Syrian civilians (Dolgin, 2013).

Chemical weapons vary in their mechanisms of action, target organs and lethality. Organophosphorus (OP) nerve agents such as sarin, soman, and VX are by far the most formidable in terms of their toxicity and lethal effects. In addition, OP pesticides, which share a common mechanism of action as OP nerve agents, are a significant threat from occupational, accidental and intentional exposures. The primary mechanism of toxicity in acute exposures to these compounds is the irreversible inhibition of acetylcholinesterase (AChE) in the nervous system and resultant over-activation of cholinergic tone via acetylcholine (ACh) accumulation. Depending on the degree and route of exposure, over-activation of cholinergic receptors can cause a myriad of clinical symptoms ranging from mild to severe. Perhaps the most prominent of the severe symptoms are seizures that rapidly progress to a period of unremitting seizure activity known as status epilepticus (SE). The sequelae of toxicity includes the early events of AChE inhibition and SE, delayed events such as cellular, metabolic and inflammatory changes and late outcomes i.e. neuropathology and long-term cognitive impairment. Increasing evidence suggests that other pathways play an important part in mediating the sequelae of toxicity resulting from such exposures. In this review, we discuss how early events lead to cellular oxidative stress and how targeting this phenomenon can alter delayed and functional outcomes (Fig. 1).

2. Redox imbalance

Oxidative stress occurs when the balance between oxidant production and antioxidant defenses is perturbed, resulting in the disruption of redox circuitry and macromolecular damage (Kemp et al., 2008). Free radicals derived from molecular oxygen and nitrogen are produced as a natural by-product of aerobic metabolism and other bodily processes by both enzymatic and non-enzymatic reactions (Turrens, 2003). The collective term, reactive species (RS), refers to oxygen radicals such as superoxide ($O_2^{\cdot-}$) and hydroxyl radicals ($\cdot OH$), oxidizing non-radicals such as hydrogen peroxide (H_2O_2), and reactive nitrogen species such as nitric oxide ($NO\cdot$) and peroxynitrite ($ONOO^-$). In the body, the major sources of RS are mitochondria, NADPH oxidases (NOX), halo-peroxidases and the nitric oxide synthases (NOS). At low levels, RS are indispensable to host defense and signal transduction. However, increasing steady-state levels of RS results in oxidative damage to cellular lipids, proteins, sugars and DNA bases with deleterious consequences to tissue health culminating in disease (Bae et al., 2011). Their production is therefore tightly regulated by the availability of endogenous antioxidants such as the superoxide dismutases (SOD), catalase, glutathione peroxidase (GPx), peroxiredoxins, and non-enzymatic antioxidants. Relative to other organs, the brain is relatively sparse in antioxidant defenses. This, together with the abundance of polyunsaturated lipids and high metabolic demand makes the brain particularly susceptible to RS-induced damage. Under physiologically low levels of RS, neurons are typically able to minimize RS-induced damage to cellular macromolecules through available antioxidant defenses or repair processes. Under pathological conditions when RS production is excessive and/or antioxidant and repair processes may be compromised, cellular damage causing altered cellular function can result. Specifically, damage to membrane lipids can lead to altered membrane fluidity, permeability,

and structure of membrane proteins which can in turn alter neurotransmitter uptake and release in addition to the maintenance of proper ionic gradients (Wong-ekkabut et al., 2007). Oxidative damage to cellular proteins can alter their structure and function, thereby increasing their susceptibility to proteolytic degradation and disturbing signaling pathways (Stadtman and Levine, 2000). When produced in close enough proximity, RS can damage nuclear or mitochondrial DNA. *If oxidative DNA damage exceeds what can properly be repaired by excision repair processes, the result is activation of cell death pathways* (Duprez et al., 2009; Ott et al., 2007). Neuronal cell death and inflammation are hallmark features of nerve agent toxicity and recent evidence suggests that targeting oxidative stress can attenuate these processes (Liang et al., 2018a; Liang et al., 2018b; Zaja-Milatovic et al., 2009).

3. Sources of RS formation following OP nerve agent exposure

The earliest event that occurs following OP nerve agent exposure is accumulation of the toxicant in target tissues. This is followed rapidly by the inhibition of AChE and accumulation of ACh in brain and plasma. Modest evidence suggests that mere exposure to OP toxicants is sufficient to produce signs of oxidative stress (Giordano et al., 2007; Zepeda-Arce et al., 2017). This includes studies of subacute and chronic exposures in pesticide workers where evidence of altered antioxidant status and oxidative damage is reported in the absence of AChE inhibition (Zepeda-Arce et al., 2017). Additionally, in an in vitro model of OP toxicity, indices of oxidative stress were found even in the presence of cholinergic receptor antagonists (Giordano et al., 2007). This suggests that despite the absence of AChE inhibition or ACh over-activation, oxidative stress occurs and is sufficient to result in damage, although the clinical relevance of this damage is unclear. Importantly, as subacute and chronic exposures are not associated with overt seizure activity, these studies suggest that seizure activity induced by OP nerve agent exposure is not the sole mechanism driving oxidative stress in these models. Nonetheless, most studies of subacute and chronic exposures have shown that the degree of AChE inhibition in human blood or rodent blood, liver and brain show a strong positive correlation with the appearance of oxidative stress markers in these regions (Akhgari et al., 2003; Kazi and Oommen, 2012; Ranjbar et al., 2002; Ranjbar et al., 2005). Thus, as AChE inhibition increases, so does the appearance of oxidative stress.

AChE inhibition and resultant ACh receptor hyperactivation stimulates the initiation of seizures in vulnerable brain regions (McDonough and Shih, 1993; Shih and McDonough, 1997; Shih et al., 1991). This activity is strengthened by the glutamatergic system and the resultant hyperactivation of NMDA receptors can lead to the production of RS through multiple mechanisms (Girouard et al., 2009; Gunasekar et al., 1995; Shih and McDonough, 1997). First, activation of these receptors in hippocampal CA1 neurons for example, allows for a massive influx of extracellular calcium into the cytosol (Deshpande et al., 2010; Deshpande et al., 2014). Increased intracellular calcium can promote the conversion of xanthine dehydrogenase to xanthine oxidase thereby initiating the excessive production of $O_2^{\cdot-}$ and H_2O_2 (McCord, 1985). Additionally, cytosolic calcium accumulates in mitochondria and deranges normal calcium signaling processes leading to further production of RS. Indeed, exposure to OPs has been shown to result in sustained elevations of intracellular calcium, achieved initially through NMDA receptors but maintained for up

to weeks following exposure by calcium-induced calcium release from the endoplasmic reticulum (Deshpande et al., 2010; Deshpande et al., 2014). Inhibition of this calcium plateau with dantrolene is neuroprotective in the paraxon model of OP toxicity suggesting its potential clinical relevance (Deshpande et al., 2016a; Deshpande et al., 2016b). It is possible that this protection may be in part due to dantrolene's effect on oxidative stress (Büyükkokuro lu et al., 2008). This suggests a potentially important relationship between initial NMDA receptor activation, elevated intracellular calcium, oxidative stress and neurodegeneration. Another consequence of activation of NMDA receptors or excessive intracellular calcium is the production of NO (Bredt, 1999; Garthwaite et al., 1989; Girouard et al., 2009). NO reacts with $O_2^{\cdot-}$ to form the highly toxic ONOO⁻ (Beckman and Koppenol, 1996). Increasing evidence suggests that targeting NO production may have anti-seizure and neuroprotective effects in OP models (Gupta et al., 2001b; Kim et al., 1997; Kim et al., 1999). Thus, activation of NMDA receptors sets into motion multiple pathways leading to the production and exacerbation of RS.

Another major cause of RS formation upon exposure to toxic levels of OPs are the seizures, most commonly continuous seizures or status epilepticus (SE), they elicit. During a seizure, cerebral metabolism is increased to meet the bioenergetic demands of synchronous neuronal firing (Engel et al., 1982a, b). Since a single episode of OP exposure is sufficient to result in chronic epilepsy, it is plausible that the underlying epileptogenic insult also shares the metabolic signatures observed in epilepsies from other etiologies (De Araujo Furtado et al., 2010; Kadar et al., 1992). One common metabolic signature associated with human epilepsy is an increase in glucose utilization during ictal phases followed by glucose hypometabolism during the interictal phases (Chugani et al., 1994; Engel et al., 1982, 1982; Gaillard et al., 1995). Although an increase in blood flow may compensate for any bioenergetics mismatch, these energy demands can cause an imbalance in ATP production thereby depleting high energy phosphates and impairing regulated neuronal firing (Gupta et al., 2001a; Zaja-Milatovic et al., 2009). Periods of ictal hypermetabolism followed by long periods of hypometabolism provide an ideal setting for increased steady-state levels of RS through multiple cellular sources.

Several mechanisms may contribute to increased steady-state levels of cellular $O_2^{\cdot-}$ following OP nerve agent exposure. NOX2 activation, a primary source of $O_2^{\cdot-}$, is known to increase in various models of SE (Di Maio et al., 2011; Kim et al., 2013; Patel et al., 2005; Pecorelli et al., 2015; Pestana et al., 2010). Mitochondria are also potential sources of SE-induced $O_2^{\cdot-}$ and H_2O_2 due to heightened electron leakage from the electron transport chain and transfer to molecular oxygen to form $O_2^{\cdot-}$. Additionally, SE can inhibit complex I via oxidative modification and inactivate the iron-sulfur center of mitochondrial aconitase, leading to secondary generation of $O_2^{\cdot-}$ and H_2O_2 , respectively (Liang et al., 2000; Ryan et al., 2012). Indeed, in models of chemically-induced SE and OP toxicity, $O_2^{\cdot-}$ levels are increased and contribute to neurodegeneration (Liang et al., 2000; Rieger et al., 2017). $O_2^{\cdot-}$ serves as the precursor for much more toxic RS. H_2O_2 , generated by spontaneous or enzymatic dismutation and peroxidases in the presence of ferrous iron via the Fenton reaction can produce the highly reactive HO· that readily oxidizes proteins, lipids, and DNA (Fenton, 1894; McCord and Day, 1978). $O_2^{\cdot-}$ reacts with NO to produce another highly reactive product, ONOO⁻. Both HO· and ONOO⁻ are potent oxidizing agents

and evidence suggests that these molecules are produced as a consequence of OP-induced seizures (Jacobsson et al., 1999; Kim et al., 1997; Kim et al., 1999).

Damage to cellular macromolecules and resultant neurodegeneration induced by RS is exacerbated when antioxidant defenses are inhibited. Data from this laboratory have demonstrated that acute exposure to a convulsive dose of the model organophosphate, diisopropylfluorophosphate (DFP), or the OP nerve agent, soman is sufficient to alter the ratio of oxidized to reduced glutathione (GSH) in favor of oxidized glutathione (GSSG) (Liang et al., 2018a; Liang et al., 2018b). These alterations to GSH/GSSG in addition to protein nitration, occur in a time- and brain region-dependent manner (Liang et al., 2018a; Liang et al., 2018b). Oxidative stress is apparent 24 and 48 h following exposure but not at 6 or 12 h, in vulnerable brain regions such as the hippocampus and piriform cortex but not the frontal cortex (Liang et al., 2018a; Liang et al., 2018b). In addition to serving as a specific and reliable indicator of ongoing oxidative stress in these models, the abundance of GSSG suggests a redox environment that favors oxidative stress. A common finding in most studies of OP toxicity in the brain is alterations to endogenous antioxidant systems (Brocardo et al., 2007; Brocardo et al., 2005; Giordano et al., 2007; Gupta et al., 1992; Kaur et al., 2007; Lukaszewicz-Hussain, 2008; Trevisan et al., 2008; Tüzmen et al., 2007). Interestingly, alterations to brain antioxidant systems are not specific to acute exposures but encompass subconvulsive and chronic exposures (Lukaszewicz-Hussain, 2008; Slotkin et al., 2007; Trevisan et al., 2008; Tüzmen et al., 2007). This suggests that seizures are not necessarily the only driving force behind OP-induced oxidative stress.

Secondary to toxicant accumulation in target tissues, receptor activation and seizures, exposure to nerve agents results in extensive neuropathology in vulnerable brain regions. Gliosis and neuronal degeneration begin to appear in a region dependent manner starting as early as 4 to 8 h following exposure (Li et al., 2011). By 24 h after exposure, neuronal degeneration is apparent in the amygdala, hippocampus, piriform cortex and thalamus and largely co-localizes with TUNEL staining, a marker of apoptotic cell death (Li et al., 2011). Oxidative stress and neuroinflammation, known contributors to neurodegeneration also rise dramatically during this time. In the DFP and soman models, significant oxidative damage is observed in the piriform cortex and hippocampus, two particularly vulnerable brain regions, as early as 12 to 24 h following exposure (Klaidman et al., 2003; Liang et al., 2018a; Liang et al., 2018b; Pazdernik et al., 2001). Markers of neuroinflammation such as gliosis or increased levels of pro-inflammatory cytokines in these models typically dramatically increase at 24 h and can persist for weeks following exposure (Li et al., 2015; Li et al., 2011; Liang et al., 2018a; Liang et al., 2018b). Pro-inflammatory cytokines and chemokines can induce RS production and conversely, excessive RS production can induce inflammation. Therefore, the persistent neuroinflammation and oxidative stress seen in these models converge in a feed forward cycle, accumulating cellular damage leading to both acute and delayed neuropathology. Given that this neuropathology occurs in brain regions imperative to normal cognitive function, damage to these areas is likely to confer functional deficits in cognition and emotionality. Indeed, survivors of nerve agent exposures often have some degree of cognitive dysfunction such as deficits in learning and memory and are more likely to have psychiatric disorders (Dolgin, 2013; Miyaki et al., 2005; Proctor et al., 2006). Neuropathology could therefore be thought of as a surrogate to, or indicative of, functional

outcomes. Evidence supporting this is beginning to emerge in the literature (Flannery et al., 2016). Medical countermeasures that attenuate or inhibit neuropathology likely confer at least some protection against cognitive and psychiatric impairment (Pearson et al., 2015).

4. Antioxidant strategies

Due to the accumulating evidence that oxidative stress plays an important part in mediating OP and nerve-agent induced toxicity, the potential to target RS pharmacologically has attracted growing interest (Table 1). Among many available strategies, the most common is to mitigate oxidative stress in these models using small molecule antioxidant compounds with drug-like properties (Patel, 2016). Examples of small molecule antioxidants that scavenge RS in a stoichiometric manner include α -tocopherol (vitamin E or its water-soluble form, Trolox), ascorbate (vitamin C) and thiols (e.g. *N*-acetyl cysteine or glutathione esters). Catalytic mechanisms can be ascribed to non-metal (e.g. nitroxides and fullerenes) or metal based (e.g. metalloporphyrins and salens) compounds. Vitamin E is lipid soluble dietary antioxidant, which can access the blood brain barrier and inhibit brain lipid peroxidation. In the *DFP and carbofuran rat* models of OP toxicity, treatment with Vitamin E attenuated ATP depletion, oxidative damage and neurodegeneration (Gupta et al., 2001a; Gupta et al., 2001b; Jaiswal et al., 2014; Zaja-Milatovic et al., 2009). Vitamin E interacts with lipid peroxy radicals stoichiometrically, or on a 1:1 basis, it therefore needs to be present in large amounts and in combination with ascorbate to successfully prevent membrane oxidative stress and resultant lipid damage (Matsuo et al., 1989). Additionally, in the above mentioned studies, Vitamin E was given as a pre-treatment which is typically not feasible in the case of accidental or intentional exposure of civilians to *OP nerve agents or pesticides*.

Catalytic antioxidants, on the other hand, contain redox-active metal centers that catalyze the dismutation reaction of RS similar to the ability of endogenous antioxidants, allowing for regeneration of the parent compound which increases efficacy and reduces the dosage required for protection (Day, 2004). These molecular mimetics of SOD and catalase therefore hold particular promise to prevent free-radical mediated damage. Manganese (III) meso-tetrakis (di-*N*-ethylimidazole) porphyrin or Mn^{III}TDE-2-ImP⁵⁺ (also denoted in the literature as AEOL 10150) is one such metalloporphyrin antioxidant which has been shown to catalytically remove O₂^{•-}, H₂O₂, lipid peroxides and ONOO⁻ (Day et al., 1999; Kachadourian et al., 2004). It possesses a manganese moiety which functions in the dismutation reaction with O₂^{•-} by alternate reduction and oxidation in a mechanism similar to endogenous SODs. Its SOD activity exceeds that of CuZnSOD when compared on a weight basis (Kachadourian et al., 2004). In addition to the Mn center, the extensive conjugated ring system of Mn^{III}TDE-2-ImP⁵⁺ allows for reversible one-electron oxidations and likely confers its catalase-like activity of H₂O₂ detoxification (Day et al., 1997). Mn^{III}TDE-2-ImP⁵⁺, in conjunction with endogenous antioxidants, acts as a ONOO⁻ decomposition catalyst and inhibits lipid peroxidation with potent IC₅₀s (Day et al., 1999; Lee et al., 1998). Thus, Mn^{III}TDE-2-ImP⁵⁺ combines the catalytic efficiency of endogenous antioxidants with the broad spectrum of reactivity against several biologically important RS. Together, these features make it an ideal candidate therapeutic to test the role of oxidative stress initiated by OP exposures. In the following section, the effects of Mn^{III}TDE-2-ImP⁵⁺

are described as an example of one redox based therapeutic to counteract the toxicity of surrogate nerve agents (pilocarpine or DFP) and soman.

In the soman and DFP models of cholinergic toxicity, Mn^{III}TDE-2-ImP⁵⁺ has demonstrated favorable pharmacokinetics in both plasma and brain (Liang et al., 2018a; Liang et al., 2018b). Mn^{III}TDE-2-ImP⁵⁺ accesses the blood brain barrier at concentrations shown to be clinically relevant in control animals and in those exposed to soman the nerve agent surrogates DFP and pilocarpine (Liang et al., 2018a; Liang et al., 2018b; Pearson et al., 2015). In the pilocarpine rat model, when injected one hour following administration of SE and every 4 h thereafter, Mn^{III}TDE-2-ImP⁵⁺ significantly attenuated mortality, hippocampal oxidative stress, neurodegeneration, and learning and memory impairment (Pearson-Smith et al., 2017; Pearson et al., 2015). Importantly, an analysis of EEG power in groups treated with vehicle or Mn^{III}TDE-2-ImP⁵⁺ revealed no effect of drug treatment on the intensity or duration of SE, thus suggesting that the beneficial effects of the compound can be directly attributed to its antioxidant capabilities (Pearson et al., 2015). Similarly, in the DFP model, treatment with Mn^{III}TDE-2-ImP⁵⁺ did not affect brain or plasma AChE activity, suggesting that it does not act as a direct scavenger of DFP or otherwise alter AChE activity (Liang et al., 2018b). Therefore, Mn^{III}TDE-2-ImP⁵⁺ not only has therapeutic potential in OP toxicity but can also serve as a tool to probe the role of oxidative stress in OP nerve agent models.

Given the pharmacokinetic profile of Mn^{III}TDE-2-ImP⁵⁺ in surrogate and nerve agent models, comprehensive studies were performed to determine the optimal therapeutic window of the compound. In the pilocarpine model, Mn^{III}TDE-2-ImP⁵⁺ significantly attenuated indices of oxidative stress in both pre-exposure (30 min before pilocarpine) and post-exposure (60 or 90 min after pilocarpine) treatment paradigms (Pearson-Smith et al., 2017; Pearson et al., 2015). Although neuropathology was not investigated in the pretreatment paradigm, treatment with Mn^{III}TDE-2-ImP⁵⁺ when initiated 60 min following pilocarpine significantly attenuated inflammatory cytokine production, microgliosis, mTOR activation, neurodegeneration and cognitive dysfunction (McElroy et al., 2017; Pearson et al., 2015). When treatment with the compound was initiated 90 min after pilocarpine, microgliosis and neurodegeneration were modestly but significantly attenuated (Pearson-Smith et al., 2017). Thus, in the pilocarpine model, the therapeutic window of Mn^{III}TDE-2-ImP⁵⁺ extends up to 90 min post-exposure, with optimal protection exerted when treatment with the compound is initiated 60 min after pilocarpine. In the DFP and soman rat models, treatment with Mn^{III}TDE-2-ImP⁵⁺ significantly attenuated indices of oxidative stress in the hippocampus and piriform cortex when given 1, 5, or 15 min after the initiation of SE (Liang et al., 2018a; Liang et al., 2018b). Optimal protection against neuroinflammation and neurodegeneration in these models was observed when treatment with the compound began 5 min after SE initiation. SE typically develops within 10 min following exposure to DFP or soman, thus, the therapeutic window for Mn^{III}TDE-2-ImP⁵⁺ in these models extends up to 25 min following exposure. It is perhaps unsurprising that the therapeutic window of Mn^{III}TDE-2-ImP⁵⁺ shortens as the toxicity and perhaps intensity of SE induced by the toxicant increases. Of note, all rats in the above-mentioned studies received the same dose of toxicant, which would be virtually impossible in a real world scenario of chemical weapons exposure. In the case of an actual incident, people would be exposed to varying doses of the toxicant depending on a number of factors, including the proximity to the release of

the toxicant. Consequently, victims would present along a spectrum of toxicity and the therapeutic window of Mn^{III}TDE-2-ImP⁵⁺ would vary by person. Victims exposed to the highest doses would presumably have the shortest therapeutic window while those exposed to lower doses would have the longest therapeutic windows.

The utility of Mn^{III}TDE-2-ImP⁵⁺ extends beyond OP exposures with the ability to limit toxicity resulting from other chemical agents in post-exposure treatment paradigms. Mn^{III}TDE-2-ImP⁵⁺ has been demonstrated to improve mortality and inhibit lung, skin and nasal damage resulting from exposure to the vesicating and alkylating agent, sulfur mustard and its analog, 2-chloroethyl ethyl sulfide (CEES) (McElroy et al., 2016; O'Neill et al., 2011; O'Neill et al., 2010; Tewari-Singh et al., 2014). The compound was also found to protect the lung from exposure to chlorine gas (McGovern et al., 2011). Similarly, Mn^{III}TDE-2-ImP⁵⁺ improved mortality and mitigated lung damage resulting from an acute fatal dose of radiation in non-human primates (Garofalo et al., 2014; MacVittie et al., 2017). Sulfur mustard, chlorine and acute doses of radiation induce an acute respiratory distress syndrome (ARDS) that can be fatal and for which there are currently no U.S. Food and Drug Administration (FDA)-approved mitigating drugs. The Medical Imaging Products Division of the FDA has therefore granted Mn^{III}TDE-2-ImP⁵⁺ Fast Track designation as a radioprotectant (IND#112,103). Thus, Mn^{III}TDE-2-ImP⁵⁺, and perhaps antioxidant therapies in general, may be useful in situations where exposure to a chemical or radioactive weapon is suspected but the identity of the weapon is unknown or in the case of suspected use of more than one chemical weapon.

An unfortunate real world example of the practicality of Mn^{III}TDE-2-ImP⁵⁺ as a treatment for suspected chemical weapons exposure comes from the April 2017 attack on civilians in Khan Shaykhun, Syria, which left several dozen dead and hundreds injured (Gulland, 2017). Victims presented with pinpoint pupils and muscle spasms, consistent with exposure to a nerve agent such as sarin. Other victims smelt of bleach, indicative of exposure to chlorine. Médecins Sans Frontières later determined that at least two chemical weapons were used. In a situation such as this, a broad spectrum countermeasure such as Mn^{III}TDE-2-ImP⁵⁺ could be administered without prior knowledge regarding the nature of the toxicant. Mn^{III}TDE-2-ImP⁵⁺ is stable at room temperature for at least two years (stability testing is ongoing) in light resistant containers so storage in ambulances or a hospital setting is feasible (Zhang et al., 2018). It may be used at the site, ambulance or in the hospital setting as it targets late effects; however, earlier intervention with daily injectable dosing would be optimal. In animals, Mn^{III}TDE-2-ImP⁵⁺ was effective at protecting the lung and skin when given at 5 mg/kg up to one hour after exposure to sulfur mustard or CEES (McElroy et al., 2016; O'Neill et al., 2011; O'Neill et al., 2010; Tewari-Singh et al., 2014). Doses ranging from 5 to 7 mg/kg were sufficient to protect the brain from nerve agents and their surrogates when given 15 min to one hour following exposure (Liang et al., 2018a; Liang et al., 2018b; Pearson et al., 2015). In mice, Mn^{III}TDE-2-ImP⁵⁺ was well tolerated with a no observable adverse effect level (NOAEL) of 40 mg/kg/day and a maximum tolerated dose (MTD) of 160 mg/kg/day (McGovern et al., 2011). In human clinical studies, Mn^{III}TDE-2-ImP⁵⁺ was safe and well tolerated in 9 healthy subjects receiving ascending single doses (25, 50 and 75 mg), 12 patients with Amyotrophic Lateral Sclerosis (ALS) receiving multiple doses over 7 days and 28 ALS patients receiving a single dose (Zhang et al., 2018). Thus,

Mn^{III}TDE-2-ImP⁵⁺ has been shown to be safe in human studies and can protect multiple organ systems from the toxic effects of at least two classes of chemical warfare agents. Based on the available data and information, further development of antioxidant compounds such as Mn^{III}TDE-2-ImP⁵⁺ as medical countermeasures is warranted.

5. Limitations of antioxidant compounds in treating OP nerve agent toxicity

Although we provide a strong rationale for antioxidant therapies in OP nerve agent toxicity, several barriers to effective treatment must also be considered. First, any potential therapeutic compound must possess rapid entry and maintain sufficient concentrations in the brain to counteract the CNS effects of nerve agent exposure. While Mn^{III}TDE-2-ImP⁵⁺ is able to reach the rodent brain within ~15 min (unpublished data; Patel laboratory), whether other antioxidant compounds can achieve sufficient concentrations in varying post-exposure periods must be established. One of the major limitations of natural antioxidants such as Vitamins E and C are their large size, which limits cell permeability. To overcome this, SOD mimetics such as Mn^{III}TDE-2-ImP⁵⁺ are generally of low molecular weight, allowing for improved access to the brain and intracellular compartments. Delivery of therapeutics past the blood brain barrier (BBB) is also likely aided by increased BBB permeability caused by seizure activity (Gupta et al., 1999; Han et al., 2017; Song et al., 2004). Additionally, a newer class of mitochondria-targeted antioxidants such as mitoquinone mesylate (MitoQ) and its analogs have been developed by covalently binding ubiquinone to a triphenylphosphonium (TPP⁺) cation (Kelso et al., 2001). This allows for the delivery of antioxidants to the mitochondria, the major site of RS production at concentrations far exceeding what has been previously possible. One caveat of these compounds is that their uptake is dependent upon an active mitochondrial membrane potential which may or may not be affected in nerve agent exposures. Whether mitochondria-targeted antioxidant therapy confers any added protection over SOD mimetics in nerve agent toxicity remains to be elucidated.

Another challenge to antioxidants as an add-on neuroprotective strategy for OP nerve agent toxicity is the time course of oxidative stress in these models. We have demonstrated that oxidative stress extends beyond the first 24 h following OP or nerve agent exposure (Liang et al., 2018a; Liang et al., 2018b). It is therefore necessary to administer antioxidant therapy over extended periods to mitigate oxidative damage. Indeed, one of the major limitations of Mn^{III}TDE-2-ImP⁵⁺ is its short half-life which necessitates injections every four hours. While repeated administration of therapeutics is feasible in a hospital setting, antioxidant compounds with a longer half-life necessitating fewer injections would be ideal. Finally, safety studies of the compound alone or in combination with other adjuvants would be needed to guide future development.

6. Concluding remarks

There are multiple shared mechanisms by which redox imbalance and oxidative stress occurs following exposure to chemical warfare agents. In models of OP toxicity, oxidative stress regardless of its source, can lead to secondary neuronal injury. Antioxidant treatment

strategies therefore, represent a novel and promising approach to target secondary neuronal injury and exert neuroprotection.

Acknowledgements

R21NS072099, U01NS083422 and NS039587, NS039587-S1

References

- Akhgari M, et al. , 2003. Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. *Human & Experimental Toxicology* 22, 205–211. [PubMed: 12755471]
- Bae YS, et al. , 2011. Regulation of reactive oxygen species generation in cell signaling. *Molecules and cells* 32, 491–509. [PubMed: 22207195]
- Beckman JS, Koppenol WH, 1996. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am. J. Phys. Cell Phys* 271, C1424–C1437.
- Bredt DS, 1999. Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Rad. Res* 31.
- Brocardo PS, et al. , 2005. Antioxidant defenses and lipid peroxidation in the cerebral cortex and hippocampus following acute exposure to malathion and/or zinc chloride. *Toxicology* 207, 283–291. [PubMed: 15596258]
- Brocardo PS, et al. , 2007. Zinc attenuates Malathion-induced depressant-like behavior and confers Neuroprotection in the rat brain. *Toxicol. Sci* 97, 140–148. [PubMed: 17327255]
- Büyükkokuro lu ME, et al. , 2008. Dantrolene may prevent organophosphate-induced oxidative stress and muscle injury. *Pestic. Biochem. Physiol* 92, 156–163.
- Chugani HT, et al. , 1994. Ictal patterns of cerebral glucose utilization in children with epilepsy. *Epilepsia* 35, 813–822. [PubMed: 8082628]
- Day BJ, 2004. Catalytic antioxidants: a radical approach to new therapeutics. *Drug Discov. Today* 9, 557–566. [PubMed: 15203091]
- Day BJ, et al. , 1997. Manganic Porphyrins possess catalase activity and protect endothelial cells against hydrogen peroxide-mediated injury. *Arch. Biochem. Biophys* 347, 256–262. [PubMed: 9367533]
- Day BJ, et al. , 1999. Metalloporphyrins are potent inhibitors of lipid peroxidation. *Free Radic. Biol. Med* 26, 730–736. [PubMed: 10218663]
- De Araujo Furtado M, et al. , 2010. Spontaneous recurrent seizures after status epilepticus induced by soman in Sprague-Dawley rats. *Epilepsia* 51, 1503–1510. [PubMed: 20067510]
- Deshpande LS, et al. , 2010. Development of a prolonged calcium plateau in hippocampal neurons in rats surviving status Epilepticus induced by the organophosphate Diisopropylfluorophosphate. *Toxicol. Sci* 116, 623–631. [PubMed: 20498005]
- Deshpande LS, et al. , 2014. Development of status epilepticus, sustained calcium elevations and neuronal injury in a rat survival model of lethal paraoxon intoxication. *Neurotoxicology* 44, 17–26. [PubMed: 24785379]
- Deshpande LS, et al. , 2016a. Pharmacological blockade of the calcium plateau provides neuroprotection following organophosphate paraoxon induced status epilepticus in rats. *Neurotoxicol. Teratol* 56, 81–86. [PubMed: 27224207]
- Deshpande LS, et al. , 2016b. Role of the calcium plateau in neuronal injury and behavioral morbidities following organophosphate intoxication. *Ann. N. Y. Acad. Sci* 1374, 176–183. [PubMed: 27327161]
- Di Maio R, et al. , 2011. Pilocarpine alters NMDA receptor expression and function in hippocampal neurons: NADPH oxidase and ERK1/2 mechanisms. *Neurobiol. Dis* 42, 482–495. [PubMed: 21397025]
- Dolgin E, 2013. Syrian gas attack reinforces need for better anti-sarin drugs. *Nat. Med* 19, 1194. [PubMed: 24100968]

- Duprez L, et al. , 2009. Major cell death pathways at a glance. *Microbes Infect.* 11, 1050–1062. [PubMed: 19733681]
- Engel J, et al. , 1982a. Patterns of human local cerebral glucose metabolism during epileptic seizures. *Science* 218, 64–66. [PubMed: 6981843]
- Engel J Jr., et al. , 1982b. Pathological findings underlying focal temporal lobe hypometabolism in partial epilepsy. *Ann. Neurol* 12, 518–528. [PubMed: 6984318]
- Fenton HJH, 1894. LXXIII.-oxidation of tartaric acid in presence of iron. *J. Chem. Soc. Trans* 65, 899–910.
- Flannery BM, et al. , 2016. Persistent neuroinflammation and cognitive impairment in a rat model of acute diisopropylfluorophosphate intoxication. *J. Neuroinflammation* 13, 267. [PubMed: 27733171]
- Gaillard WD, et al. , 1995. FDG-PET in children and adolescents with partial seizures: role in epilepsy surgery, evaluation. *Epilepsy Res.* 20, 77–84. [PubMed: 7713062]
- Garofalo MC, et al. , 2014. A pilot study in rhesus macaques to assess the treatment efficacy of a small molecular weight catalytic Metalloporphyrin antioxidant (AEOL 10150) in mitigating radiation-induced lung damage. *Health Phys.* 106, 73–83. [PubMed: 24276551]
- Garthwaite J, Garthwaite G, Palmer RM, et al. , 1989. NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. *Eur. J. Pharmacol* 172, 413–416.
- Giordano G, et al. , 2007. Organophosphorus insecticides chlorpyrifos and diazinon and oxidative stress in neuronal cells in a genetic model of glutathione deficiency. *Toxicol. Appl. Pharmacol* 219, 181–189. [PubMed: 17084875]
- Girouard H, et al. , 2009. NMDA receptor activation increases free radical production through nitric oxide and NOX2. *J. Neurosci* 29, 2545–2552. [PubMed: 19244529]
- Gulland A, 2017. WHO condemns suspected use of chemical weapons. *BMJ* 357.
- Gunasekar PG, et al. , 1995. NMDA receptor activation produces concurrent generation of nitric oxide and reactive oxygen species: implications for cell death. *J. Neurochem* 65, 2016–2021. [PubMed: 7595485]
- Gupta J, Datta C, Sarkar A, et al. , 1992. Effect of malathion on antioxidant defense system in human fetus - an in vitro study. *Indian J. Exp. Biol* 30, 352–354. [PubMed: 1459609]
- Gupta A, et al. , 1999. Functional impairment of blood-brain barrier following pesticide exposure during early development in rats. *Human & Experimental Toxicology* 18, 174–179.
- Gupta RC, et al. , 2001a. Depletion of energy metabolites following Acetylcholinesterase inhibitor-induced status Epilepticus: protection by antioxidants. *NeuroToxicology* 22, 271–282. [PubMed: 11405258]
- Gupta RC, et al. , 2001b. Nitric oxide modulates high-energy phosphates in brain regions of rats intoxicated with diisopropylphosphorofluoridate or carbofuran: prevention by N-tert-butyl-alpha-phenylnitron or vitamin E. *Arch. Toxicol* 75, 346–356. [PubMed: 11570692]
- Han H, et al. , 2017. Breaking bad: the structure and function of the blood-brain barrier in epilepsy. *AAPS J.* 19, 973–988. [PubMed: 28550637]
- Jacobsson SO, et al. , 1999. Increased levels of nitrogen oxides and lipid peroxidation in the rat brain after soman-induced seizures. *Arch. Toxicol* 73, 269–273. [PubMed: 10463393]
- Jaiswal SK, et al. , 2014. Carbofuran induced oxidative stress mediated alterations in Na⁺-K⁺-ATPase activity in rat brain: amelioration by vitamin E. *J. Biochem. Mol. Toxicol* 28, 320–327. [PubMed: 24865641]
- Jett DA, Yeung DT, 2010. The CounterACT research network: basic mechanisms and practical applications. *Proc. Am. Thorac. Soc* 7, 254–256. [PubMed: 20601628]
- Kachadourian R, et al. , 2004. Flavin-dependent antioxidant properties of a new series of meso-N,N'-dialkyl-imidazolium substituted manganese(III) porphyrins. *Biochem. Pharmacol* 67, 77–85. [PubMed: 14667930]
- Kadar T, et al. , 1992. Long-term study of brain lesions following Soman, in comparison to DFP and Metrazol poisoning. *Human & Experimental Toxicology* 11, 517–523. [PubMed: 1361142]

- Kaur P, et al. , 2007. Impaired mitochondrial energy metabolism and neuronal apoptotic cell death after chronic dichlorvos (OP) exposure in rat brain. *NeuroToxicology* 28, 1208–1219. [PubMed: 17850875]
- Kazi AI, Oommen A, 2012. Monocrotophos induced oxidative damage associates with severe acetylcholinesterase inhibition in rat brain. *NeuroToxicology* 33, 156–161. [PubMed: 22285544]
- Kelso GF, et al. , 2001. Selective targeting of a redox-active ubiquinone to mitochondria within cells: ANTIOXIDANT AND ANTIAPOPTOTIC PROPERTIES. *J. Biol. Chem* 276, 4588–4596. [PubMed: 11092892]
- Kemp M, et al. , 2008. Non-equilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems biology. *Free Radic. Biol. Med* 44, 921–937. [PubMed: 18155672]
- Kim Y-B, et al. , 1997. A role of nitric oxide in organophosphate-induced convulsions. *Environ. Toxicol. Pharmacol* 3, 53–56. [PubMed: 21781758]
- Kim Y-B, et al. , 1999. Organophosphate-induced brain injuries: delayed apoptosis mediated by nitric oxide. *Environ. Toxicol. Pharmacol* 7, 147–152. [PubMed: 21781920]
- Kim JH, et al. , 2013. Post-treatment of an NADPH oxidase inhibitor prevents seizure-induced neuronal death. *Brain Res.* 1499, 163–172. [PubMed: 23313582]
- Klaidman LK, et al. , 2003. Alterations in brain glutathione homeostasis induced by the nerve gas soman. *Neurotox. Res* 5, 177–182. [PubMed: 12835122]
- Lee J, et al. , 1998. Manganese Porphyrins as redox-coupled Peroxynitrite Reductases. *J. Am. Chem. Soc* 120, 6053–6061.
- Li Y, et al. , 2011. Spatiotemporal pattern of neuronal injury induced by DFP in rats: a model for delayed neuronal cell death following acute OP intoxication. *Toxicol. Appl. Pharmacol* 253, 261–269. [PubMed: 21513723]
- Li Y, et al. , 2015. Neuregulin-1 inhibits neuroinflammatory responses in a rat model of organophosphate-nerve agent-induced delayed neuronal injury. *J. Neuroinflammation* 12, 64. [PubMed: 25880399]
- Liang LP, et al. , 2000. Mitochondrial superoxide production in kainate-induced hippocampal damage. *Neuroscience* 101, 563–570. [PubMed: 11113305]
- Liang L-P, et al. , 2018a. Neuroprotective effects of a catalytic antioxidant in a rat nerve agent model. *Redox Biol.* 20, 275–284. [PubMed: 30384261]
- Liang L-P, et al. , 2018b. Neuroprotective effects of AEOL10150 in a rat organophosphate model. *Toxicol. Sci* 162, 611–621. [PubMed: 29272548]
- Lukaszewicz-Hussain A, 2008. Subchronic intoxication with chlorfenvinphos, an organophosphate insecticide, affects rat brain antioxidative enzymes and glutathione level. *Food Chem. Toxicol* 46, 82–86. [PubMed: 17706853]
- MacVittie TJ, et al. , 2017. AEOL 10150 mitigates radiation-induced lung injury in the nonhuman primate: morbidity and mortality are administration schedule-dependent. *Radiat. Res* 187, 298–318. [PubMed: 28208025]
- Matsuo M, et al. , 1989. Radical-scavenging reactions of vitamin E and its model compound, 2,2,5,7,8-pentamethylchroman-6-ol, in a tert-butylperoxyl radical generating system. *J. Am. Chem. Soc* 111, 7179–7185.
- McCord JM, 1985. Oxygen-derived free radicals in postischemic tissue injury. *N. Engl. J. Med* 312, 159–163. [PubMed: 2981404]
- McCord JM, Day ED, 1978. Superoxide-dependent production of hydroxyl radical catalyzed by iron—EDTA complex. *FEBS Lett.* 86, 139–142. [PubMed: 202505]
- McDonough JH, Shih T-M, 1993. Pharmacological modulation of soman-induced seizures. *Neurosci. Biobehav. Rev* 17, 203–215. [PubMed: 8515903]
- McElroy CS, et al. , 2016. From the cover: catalytic antioxidant Rescue of Inhaled Sulfur Mustard Toxicity. *Toxicological sciences : an official journal of the Society of Toxicology* 154, 341–353. [PubMed: 27605419]
- McElroy PB, et al. , 2017. Scavenging reactive oxygen species inhibits status epilepticus-induced neuroinflammation. *Exp. Neurol* 298, 13–22. [PubMed: 28822838]

- McGovern T, et al. , 2011. AEOL10150: a novel therapeutic for rescue treatment after toxic gas lung injury. *Free Radic. Biol. Med* 50, 602–608. [PubMed: 21156205]
- Miyaki K, et al. , 2005. Effects of sarin on the nervous system of subway workers seven years after the Tokyo subway sarin attack. *J. Occup. Health* 47, 299–304. [PubMed: 16096354]
- O’Neill HC, et al. , 2010. Treatment with the catalytic metalloporphyrin AEOL 10150 reduces inflammation and oxidative stress due to inhalation of the sulfur mustard analog 2-chloroethyl ethyl sulfide. *Free Radic. Biol. Med* 48, 1188–1196. [PubMed: 20138141]
- O’Neill HC, et al. , 2011. Role of reactive oxygen and nitrogen species in olfactory epithelial injury by the Sulfur mustard analogue 2-Chloroethyl ethyl Sulfide. *Am. J. Respir. Cell Mol. Biol* 45, 323–331. [PubMed: 21642592]
- Ott M, et al. , 2007. Mitochondria, oxidative stress and cell death. *Apoptosis* 12, 913–922. [PubMed: 17453160]
- Patel M, 2016. Targeting oxidative stress in central nervous system disorders. *Trends Pharmacol. Sci* 37, 768–778. [PubMed: 27491897]
- Patel M, et al. , 2005. Activation of NADPH oxidase and extracellular superoxide production in seizure-induced hippocampal damage. *J. Neurochem* 92, 123–131. [PubMed: 15606902]
- Pazdernik TL, et al. , 2001. Soman-induced seizures: limbic activity, oxidative stress and neuroprotective proteins. *J. Appl. Toxicol* 21 (Suppl. 1), S87–S94. [PubMed: 11920927]
- Pearson JN, et al. , 2015. Reactive oxygen species mediate cognitive deficits in experimental temporal lobe epilepsy. *Neurobiol. Dis* 82, 289–297. [PubMed: 26184893]
- Pearson-Smith JN, et al. , 2017. Oxidative stress contributes to status Epilepticus associated mortality. *Neurochem. Res* 42, 2024–2032. [PubMed: 28462450]
- Pecorelli A, et al. , 2015. NADPH oxidase activation and 4-hydroxy-2-nonenal/aqua-porin-4 adducts as possible new players in oxidative neuronal damage presents in drug-resistant epilepsy. *Biochim. Biophys. Acta (BBA) - Mol. Basis Dis* 1852, 507–519.
- Pestana RRF, et al. , 2010. Reactive oxygen species generated by NADPH oxidase are involved in neurodegeneration in the pilocarpine model of temporal lobe epilepsy. *Neurosci. Lett* 484, 187–191. [PubMed: 20732386]
- Proctor SP, et al. , 2006. Effects of sarin and cyclosarin exposure during the 1991 gulf war on neurobehavioral functioning in US army veterans. *Neurotoxicology* 27, 931–939. [PubMed: 16982099]
- Ranjbar A, et al. , 2002. Induction of oxidative stress and acetylcholinesterase inhibition in organophosphorous pesticide manufacturing workers. *Human & Experimental Toxicology* 21, 179–182. [PubMed: 12099619]
- Ranjbar A, et al. , 2005. Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. *Environ. Toxicol. Pharmacol* 20, 88–91. [PubMed: 21783573]
- Rieger DK, et al. , 2017. Involvement of superoxide in malaoxon-induced toxicity in primary cultures of cortical neurons. *J Toxicol Environ Health A* 80, 1106–1115. [PubMed: 28849997]
- Ryan K, et al. , 2012. Post-translational oxidative modification and inactivation of mitochondrial complex I in Epileptogenesis. *J. Neurosci* 32, 11250–11258. [PubMed: 22895709]
- Shih T-M, McDonough JH, 1997. Neurochemical mechanisms in Soman-induced seizures. *J. Appl. Toxicol* 17, 255–264. [PubMed: 9285539]
- Shih TM, et al. , 1991. Anticonvulsants for poisoning by the organophosphorus compound soman: pharmacological mechanisms. *Neurosci. Biobehav. Rev* 15, 349–362. [PubMed: 1683477]
- Slotkin TA, et al. , 2007. Screening for developmental neurotoxicity using PC12 cells: comparisons of organophosphates with a Carbamate, an Organochlorine, and divalent nickel. *Environ. Health Perspect* 115, 93–101. [PubMed: 17366826]
- Song X, et al. , 2004. Interactive effects of Paraoxon and Pyridostigmine on blood-brain barrier integrity and cholinergic toxicity. *Toxicol. Sci* 78, 241–247. [PubMed: 14976354]
- Stadtman ER, Levine RL, 2000. Protein oxidation. *Ann. N. Y. Acad. Sci* 899, 191–208. [PubMed: 10863540]

- Tayyaba K, Hasan M, 1985. Vitamin E protects against metasytox-induced adverse effect on lipid metabolism in the rat brain and spinal cord. *Acta Pharmacol Toxicol (Copenh)* 57, 190–196. [PubMed: 4061100]
- Tewari-Singh N, et al. , 2014. Catalytic antioxidant AEOL 10150 treatment ameliorates sulfur mustard analog 2-chloroethyl ethyl sulfide-associated cutaneous toxic effects. *Free Radic. Biol. Med* 72, 285–295. [PubMed: 24815113]
- Trevisan R, et al. , 2008. Antioxidant and Acetylcholinesterase response to repeated Malathion exposure in rat cerebral cortex and Hippocampus. *Basic & Clinical Pharmacology & Toxicology* 102, 365–369. [PubMed: 18341513]
- Turrens JF, 2003. Mitochondrial formation of reactive oxygen species. *J. Physiol* 552, 335–344. [PubMed: 14561818]
- Tüzmen MN, et al. , 2007. The evaluation of altered Antioxidative Defense mechanism and Acetylcholinesterase activity in rat brain exposed to Chlorpyrifos, Deltamethrin, and their combination. *Toxicol. Mech. Methods* 17, 535–540. [PubMed: 20020880]
- Wong-ekkabut J, et al. , 2007. Effect of lipid peroxidation on the properties of lipid bi-layers: a molecular dynamics study. *Biophys. J* 93, 4225–4236. [PubMed: 17766354]
- Zaja-Milatovic S, et al. , 2009. Protection of DFP-induced oxidative damage and Neurodegeneration by antioxidants and NMDA receptor antagonist. *Toxicol. Appl. Pharmacol* 240, 124–131. [PubMed: 19615394]
- Zepeda-Arce R, et al. , 2017. Oxidative stress and genetic damage among workers exposed primarily to organophosphate and pyrethroid pesticides. *Environ. Toxicol* 32, 1754–1764. [PubMed: 28233943]
- Zhang X-R, et al. , 2018. Improvements in SOD mimic AEOL-10150, a potent broad-spectrum antioxidant. *Military Medical Research* 5, 30. [PubMed: 30185231]

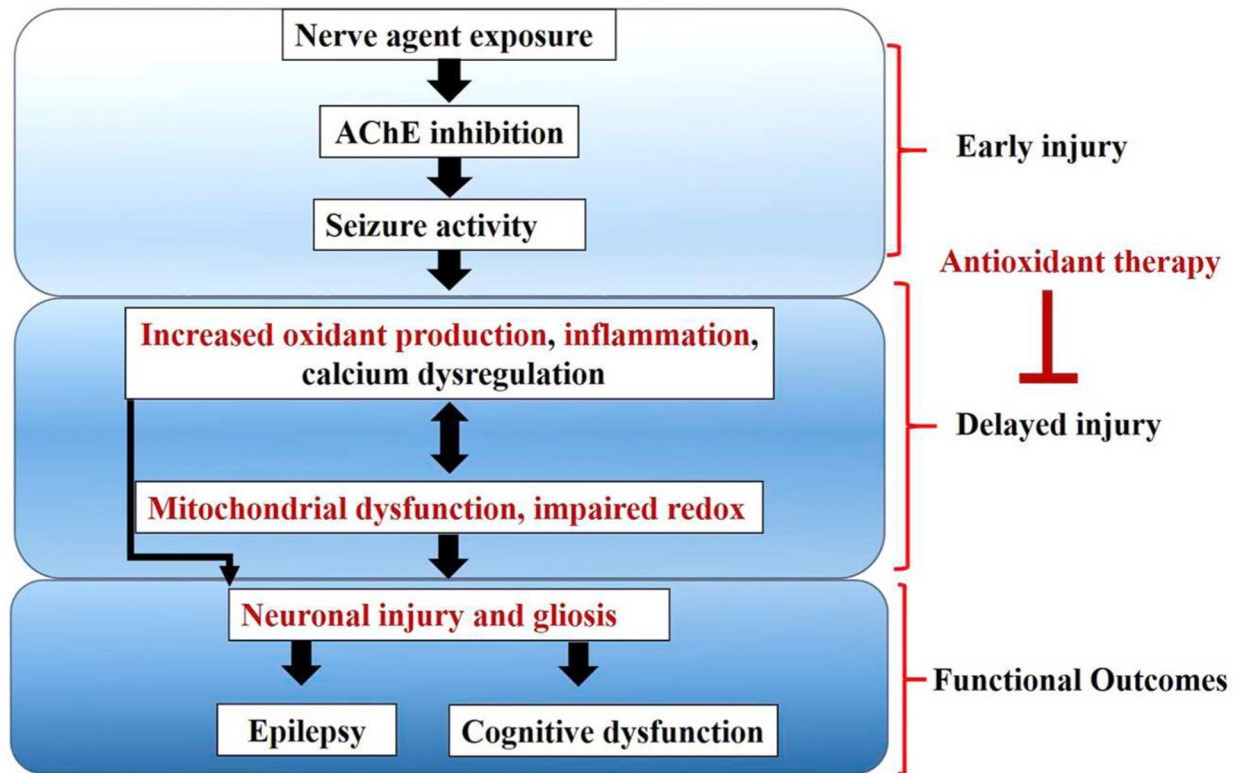


Fig. 1. Simplified schematic of how nerve agent exposure leads to delayed injury and functional outcomes. Red denotes processes shown to be inhibited by antioxidant therapy.

Table 1

Brain studies of antioxidant therapies in OP nerve agent rat models. LPO- lipid peroxidation, PBN- phenyl-alpha-tert-butyl nitron.

OP agent	Antioxidant treatment	Outcome	Citation
Demeton-methyl (Metasystox)	Vitamin E	Vit E prevented LPO	(Tayyaba and Hasan, 1985)
DFP & Carbofuran	Vitamin E and PBN	Vit E- partially prevented depletion of high energy phosphates PBN- prevented induced seizures and partially prevented depletion of high energy phosphates	(Gupta et al., 2001a; Gupta et al., 2001b; Zaja-Milatovic et al., 2009)
Carbofuran	Vitamin E	Vit E attenuated decreased SOD, catalase, GST and Na ⁺ -K ⁺ -ATPase activity	(Jaiswal et al., 2014)
DFP	Vitamin E and PBN	Both Vit E and PBN inhibited increased F ₂ -IsoPs, F ₄ -NeuroPs, and citruilline and protected dendritic length	(Zaja-Milatovic et al., 2009)
DFP	Mn ^{III} TDE-2-ImP ⁵⁺ (AEOL10150)	Attenuated neuroinflammation, neurodegeneration	(Liang et al., 2018b)
Soman	Mn ^{III} TDE-2-ImP ⁵⁺ (AEOL10150)	Attenuated neuroinflammation, neurodegeneration	(Liang et al., 2018a)