HHS Public Access

Author manuscript

Ann N Y Acad Sci. Author manuscript; available in PMC 2017 August 01.

Published in final edited form as:

Ann N Y Acad Sci. 2016 August ; 1378(1): 17–24. doi:10.1111/nyas.13115.

The role of oxidative stress in organophosphate and nerve agent toxicity

Jennifer N. Pearson[1] and **Manisha Patel**[1,2]

[1]Neuroscience Program, University of Colorado, Anschutz Medical Campus, Aurora, Colorado

[2]Department of Pharmaceutical Sciences, University of Colorado, Anschutz Medical Campus, Aurora, Colorado

Abstract

Organophosphate nerve agents exert their toxicity through inhibition of acetylcholinesterase. The excessive stimulation of cholinergic receptors rapidly causes neuronal damage, seizures, death, and long-term neurological impairment in those that survive. Owing to the lethality of organophosphorus agents and the growing risk they pose, medical interventions that prevent organophosphate toxicity and the delayed injury response are much needed. Studies have shown that oxidative stress occurs in models of subacute, acute, and chronic exposure to organophosphate agents. Key findings of these studies include alterations in mitochondrial function and increased free radical–mediated injury, such as lipid peroxidation. This review focuses on the role of reactive oxygen species in organophosphate neurotoxicity and its dependence on seizure activity. Understanding the sources, mechanisms, and pathological consequences of organophosphate-induced oxidative stress can lead to the development of rational therapies for treating toxic exposures.

Keywords

oxidative stress; organophosphates; acetylcholinesterase; nerve agents; reactive oxygen species

Introduction

Developing antidotes and countermeasures against chemical weapons is a major post–September 11th shift in research priorities initiated by U.S. research agencies. The effort to develop countermeasures against chemical threat agents spearheaded by the National Institutes of Health (NIH) Countermeasures Against Chemical Threats (CounterACT) program is particularly focused on developing countermeasure therapeutics that can be administered in a postexposure paradigm (http://www.ninds.nih.gov/research/counterterrorism/counterACT). The impetus arises from potential threats to the civilian population and/or military personnel deliberately exposed to chemical warfare agents.

Address for correspondence: Manisha Patel, Ph.D., 12850 E. Montview Blvd V20-C238, Aurora, CO. 80045. Manisha.Patel@ucdenver.edu.

Conflicts of interest

Dr. Patel is a paid consultant for Aeolus Pharma, which develops catalytic antioxidants.

Among the chemical warfare agents, organophosphate (OP) nerve agents such as VX, soman, and sarin are by far the most formidable in terms of their toxicity and lethal effects.[1] In addition, OP pesticides are a significant threat for both intentional and unintentional poisonings.

The primary mechanism of OP toxicity is the inhibition of acetylcholinesterase (AChE) in the nervous system and the resultant overactivation of cholinergic tone via acetylcholine (ACh) accumulation in synapses and neuromuscular junctions. Exposure to chemical threat agents, such as OP nerve agents/pesticides, metabolic poisons, or high levels of sulfur mustard, can trigger seizures and downstream deleterious consequences. Since the elicitation of seizures, particularly unremitting seizures known as status epilepticus (SE), is a common manifestation of OP agents that target the central nervous system, it is important for medical countermeasures to intervene at two levels.[1–4] The first level of intervention is to ameliorate the symptoms arising owing to the specific interaction of the agent and cellular targets. OP agents irreversibly bind and inhibit AChE, leading to a persistent increase in cholinergic stimulation. This produces acute effects of OP poisoning, including miosis, excessive secretions, seizures, severe muscle paralysis, cardiorespiratory depression, coma, and death.[5] Early treatment with anticholinergic drugs, such as atropine, oximes that reverse the AChE inhibition, and antiseizure drugs such as midazolam, is the primary intervention to prevent acute symptoms of toxicity.[6–8] A second level of intervention, less well understood than the first, is targeting the delayed injury response and long-term neurological consequences of exposure to these agents with neuroprotective agents.[1] In this review, we discuss evidence that suggests that oxidative stress mediates secondary OP injury and that targeting oxidative stress may be beneficial in protecting the brain and peripheral organs from the long-term consequences of OP toxicity.

Oxidative stress

Under normal physiological conditions, reactive oxygen species (ROS) are produced as by-products of aerobic metabolism by both enzymatic and nonenzymatic reactions.[9] Acceptance of a single electron by molecular oxygen results in the formation of the superoxide radical ($O_2^{\bullet -}$) which serves as the precursor of much more toxic ROS, such as hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\bullet}$). H_2O_2 is a product of $O_2^{\bullet -}$ detoxification by superoxide dismutase (SOD) and is also produced by oxidative inactivation of the iron–sulfur center of aconitase by $O_2^{\bullet -}$.[10,11] Additionally, H_2O_2 can be produced in the cell by two-electron reduction of oxygen via several dioxygenases.[9,12] H_2O_2 is detoxified by catalase and peroxidases that are also linked to glutathione (GSH) and thioredoxin/peroxiredoxin.[12,13] The contemporary definition of oxidative stress by Kemp *et al.* is “an imbalance in prooxidants and antioxidants with associated disruption of redox circuitry and macromolecular damage”.[14] The brain is particularly susceptible to oxidative damage owing to its large composition of lipids, high energy demand, and less abundant antioxidant defenses relative to other organs. Oxidative damage that overwhelms endogenous antioxidant defenses and repair processes can initiate neuronal death and gliosis.

Work from this laboratory and others has demonstrated that oxidative stress is an important consequence of chemical convulsants, such as kainic acid (KA) and the nerve agent surrogate pilocarpine.[15] Seizures induced by these chemicals are sufficient to increase indices of oxidative stress in the central nervous system. Specifically, pilocarpine-induced SE is sufficient to deplete the ratio of reduced glutathione to oxidized glutathione (GSH:GSSG) and to elevate the ratio of 3-NT/tyrosine.[16] Similar indices of oxidative stress, including evidence for increased production of superoxide and reactive nitrogen species (RNS), have been found in the other models of epileptogenesis as well, establishing oxidative stress as a common phenomenon underlying seizures.[17–19] Moreover, treatment with catalytic antioxidants has been shown to be neuroprotective after prolonged seizures, suggesting that targeting oxidative stress may be a beneficial strategy to prevent seizure-induced neuronal loss.[16,20,21] Seizure activity is the most critical injury response common to OP exposure, which raises two important questions: (1) Does OP-induced ROS formation arise due to seizure activity or from other mechanism(s)? and 2) Regardless of the source, does oxidative stress mediate OP-induced neuropathology?

Evidence for oxidative stress in OP exposure

Exposure to OP compounds produces indices of oxidative stress both *in vitro* and *in vivo. In vitro* incubation of OPs in cell models has been shown to increase the production of ROS and induce changes in endogenous antioxidant enzymes leading to free radical–mediated lipid peroxidation.[22,23] *In vivo* studies have further confirmed that oxidative stress occurs in animals exposed to a wide variety of OP compounds in varying exposure paradigms, including subacute, acute, and chronic exposures.[24] The most consistent findings across the *in vivo* brain studies are alterations in endogenous enzymatic and nonenzymatic antioxidant activity, alterations in mitochondria function, and increased free radical–mediated injury, such as lipid peroxidation.[23,25–36] Humans exposed either acutely or chronically to OP compounds develop similar indices of oxidative stress, which include decreased antioxidant capacity, free radical–mediated DNA damage, and free radical–mediated lipid peroxidation.[37–39] Thus, there is evidence to suggest that OP exposure causes oxidative stress in cells, animals, and humans.

Dependence of OP toxicity on seizures

Whether oxidative stress is caused by the seizures or merely exposure to OP agents is unknown. Studies utilizing pilocarpine exposure in rats suggest that seizure activity is required to produce oxidative stress. Specifically, rats injected with pilocarpine that did not develop continuous seizure activity or status epilepticus did not display alterations in glutathione redox status or protein nitration, suggesting that oxidative stress is seizure dependent.[16] This is also true for exposure to glutamatergic convulsants, such as KA.[40] Evidence against the seizure dependency of oxidative stress in OP models comes from subacute and chronic exposures. In subacute exposures, animals receive low doses of OP agents typically not sufficient to induce overt seizure activity or signs of severe cholinergic toxicity.[31,32,35,36] Despite a lack of overt behavioral seizures, indices of oxidative stress, such as increased lipid peroxidation and alterations in redox status, are still detected in these models.[31,32,35,36] Additional evidence comes from occupational workers exposed

chronically to low levels of OP compounds. Again, despite a lack of overt seizures, these studies show alterations in antioxidant function, increased lipid peroxidation, and increased free radical–mediated DNA damage.[37,41] Importantly, the degree of oxidative damage (i.e., lipid peroxidation) correlated with the degree of AChE inhibition in both rodents and humans, such that the greater the inhibition of AChE, the more oxidative damage was present.[37,38,42] This suggests that mild-to-moderate AChE inhibition by OP agents is sufficient to produce indices of oxidative stress without overt seizure activity. Despite these studies, it is difficult to remove the influence of increased neuronal excitation reminiscent of seizures from OP exposure. It should be pointed out that oxidative stress only arises when oxidant production overwhelms endogenous antioxidant and repair pathways, suggesting that low levels of OP exposure may be handled adequately. Moreover, electroencephalography (EEG) was not performed in these studies, and the occurrence of subclinical seizures characteristic of increased neuronal excitation cannot be ruled out.

Mechanisms of ROS production in OP toxicity

Excitotoxicity

Increasing evidence suggests that, although the cholinergic system is the main target of OP agents, the glutamatergic pathway may be responsible for at least part of the toxic effects of exposure, as glutamatergic antagonists have shown efficacy in attenuating OP-induced toxicity.[43,44] Recruitment of the glutamatergic pathway has deleterious consequences, including free radical generation and excitotoxic cell death, of which superoxide is a key mediator.[45] Glutamate activates NMDA receptors, which, upon activation, are known to increase ROS through synthesis of nitric oxide (NO).[46,47] Additionally, overactivation of glutamatergic receptors allows for excessive calcium influx into the cell, which can derange calcium dependent processes within the cell, damage mitochondria, and culminate in excitotoxic neuronal death. Indeed, exposure to diisopropyl fluorophosphate, paraoxon, and pilocarpine has been shown to result in sustained elevations of intracellular calcium.[48–50] Excessive intracellular calcium further stimulates NO production, which further increases ROS production.[51] Supporting the idea that free radical–mediated excitotoxic processes underlie neuronal loss in OP toxicity are studies showing that treatment with antioxidants and NMDA receptor antagonists dramatically attenuate indices of oxidative stress and neuronal damage following exposure to OP agents.[52,53]

Mitochondrial dysfunction

Increased intracellular calcium via activation of both cholinergic receptors and glutamate receptors results in, among other things, damage to mitochondria. Mitochondria are likely mediators of, or at least contributors to, OP-induced damage, due to the many functions they perform to maintain normal cellular processes. Of these, calcium homeostasis, cellular respiration, the production of ROS, and control of apoptotic cell death have all been reported to be altered in OP models. Interestingly, OP agents can have deleterious effects on mitochondria, even at low concentrations. Specifically, a dose of chlorpyrifos not sufficient to inhibit AChE significantly increased mitochondrial fusion versus fission events and decreased axonal mitochondria transport.[54] As mentioned previously, exposure to OP agents causes a persistent increase in intracellular calcium, which may result from impaired

mitochondrial function. Indeed, even chronic low-level exposure to dichlorvos raises mitochondrial calcium levels, impairs various complexes of the electron transport chain (ETC), decreases mitochondrial SOD, and induces apoptotic death.[30] Mitochondrial complexes I, II, III and IV of the ETC are known to be inhibited by acute exposure to OP or surrogate OP agents, and exposure to DFP has been shown to significantly deplete ATP levels within the hippocampus and amygdala.[30,55–57] Seizures create a large ATP demand and, when this is coupled with inhibition of aspects of the ETC, the result is impaired energy production and the generation of excessive ROS, specifically $O_2^{\bullet-}$ and H_2O_2. We have previously shown impaired mitochondrial bioenergetics following KA- and pilocarpine-induced seizures.[16,58] Specifically, we demonstrated that deficits in mitochondrial reserve capacity were mediated in part by ROS.[16,58] Deficits in mitochondrial respiration paired with increased ROS production cause the release of cytochrome *c* into the cytoplasm, where it initiates apoptotic death cascades. Increased cytosolic cytochrome *c* and activated caspases have been detected in models of OP toxicity, suggesting that mitochondria-mediated apoptotic events may underlie neuronal loss in these models.[30,59,60] Highlighting the importance of mitochondria in OP toxicity are studies showing mitochondrial targeted antioxidants attenuate oxidative stress and neuronal loss.[59,61]

Inhibition of endogenous antioxidant activity

A common finding in most studies of OP toxicity in the brain is alterations to endogenous antioxidant systems.[23,29,30,32–36] While different studies have found antioxidant systems such as SOD to be either increased or decreased following OP exposure, this likely reflects a number of factors, including the dose of the exposure, when the sample was taken relative to exposure, and the individual organism's ability to induce adaptive processes. Physiological activity of key endogenous antioxidants, such as GSH, SOD, and catalase, among others, is imperative to prevent oxidant-induced injury to cellular macromolecules. Studies have shown both acute and subacute exposures are sufficient to decrease activities of glutathione peroxidase, glutathione reductase, GSH, SOD, and catalase.[29,30] The role of diminished antioxidant activities on oxidative stress remains controversial given their abundance in tissues, overlapping roles, and efficiency. This is particularly true for SOD, which has extremely high reactivity and abundance in the brain, and catalase, which has a high K_m for H_2O_2.[11,62] It is uncertain whether small alterations in the activities of antioxidant enzymes in and of themselves can have effects sufficient to result in OP toxicity. However, in incidents when ROS are produced excessively (i.e., during seizures), diminished enzymatic activity of key antioxidants may contribute to OP-induced oxidative stress, which may contribute to neuronal loss. In pilocarpine-treated rats, treatment with a broad-spectrum catalytic antioxidant with SOD, catalase, and lipid peroxidation inhibitory properties attenuated neuronal loss in the hilus and CA3 regions of the hippocampus.[16] Therefore, while exogenous antioxidants may provide a viable avenue to prevent neuronal dysfunction and death following OP exposure, whether their loss following OP exposure contributes to toxicity remains to be determined.

ROS as effectors of OP pathology

Both neurons and glia represent likely sources and targets of ROS and RNS. In the KA model, 3-NT has been shown to colocalize with hippocampal principal neuronal markers.[19] Although organophosphate toxicity is most commonly associated with neuronal degeneration, increasing evidence suggests that glia cells are also affected. Dichlorvos treatment of primary microglia cells resulted in activation of microglia, as measured by increased Cd11b expression, increased NO production, and apoptotic cell death via the intrinsic mitochondria pathway, suggesting that microglia may be an important source and target of RNS.[63] Astroglia have been shown to prevent OP-induced neurite outgrowth inhibition by regulating neuronal GSH levels, highlighting a beneficial role of glia.[64] Future research would benefit from delineating the roles specific cell types play in contributing to OP-induced oxidative stress.

Given the labile, interchangeable, and reactive nature of ROS, it is difficult to identify the precise oxidant mediating damage. $O_2^{\bullet -}$, H_2O_2, RNS, and lipid peroxidation products are all known to be produced as acute consequences of chemically induced seizures.[17,19,52,65] Each of these reactive species can cause damage, including protein modification and lipid peroxidation. Given that OPs induce alterations in redox systems, once initiated there is a high likelihood of extensive damage to cellular membranes. Lipid peroxidation can have damaging consequences in the brain, as it compromises membrane structure, causing alterations in cell permeability and the activity of membrane proteins.[66] Exposure to OP agents, particularly DFP or carbofuran, are known to increase F_2-isoprostanes and F_4-neuroprostanes, two biomarkers of lipid peroxidation, the latter of which is associated with neuronal-specific lipid peroxidation.[52,53] DFP-induced F_4-neuroprostane production has been associated with dendritic degeneration of pyramidal neurons in the CA1 region of the hippocampus, which was completely attenuated by treatment with the antioxidants vitamin E or PBN.[53] Given that lipid peroxidation can be initiated by a wide variety of ROS, perhaps the best way to prevent damage is by use of a broad-spectrum antioxidant.

Oxidative stress as a therapeutic target

Endogenous antioxidant mimetics

Catalytic antioxidants contain redox-active metal centers that catalyze the dismutation reaction of ROS, similar to the ability of endogenous antioxidants.[67] These molecular mimetics of SOD, glutathione peroxidase, or catalase are potent inhibitors of $O_2^{\bullet -}$, H_2O_2, lipid peroxides, and $ONOO^-$ that hold particular promise to prevent free radical–mediated damage.[67] Because they are catalytic, and not merely free radical scavengers, these compounds are much more potent antioxidants than dietary additives, such as vitamin E, that act stoichiometrically. The manganese mesoporphyrin catalytic antioxidant, Mn^{III}TDE-2-ImP^{5+}, often denoted as AEOL10150, combines the broad spectrum of reactivity like the stoichiometric antioxidants with the catalytic efficiency of the endogenous antioxidant enzymes. AEOL10150 is a prototypical water-soluble metalloporphyrin that possesses extremely high SOD activity. On a weight basis, its SOD activity surpasses that of CuZnSOD. It also catalyzes the dismutation of H_2O_2 and inhibits lipid peroxidation with potent IC50s and scavenges $ONOO^-$ efficiently.[68–70] Treatment with AEOL10150 prevented

pilocarpine-induced mitochondrial dysfunction, as measured by maximal respiratory rates, ATP turnover, baseline respiration, and glycolytic rates.[16] In the same study, treatment with AEOL10150 attenuated neuronal loss within the hippocampus and prevented deficits in spatial memory and recognition memory, attesting to the efficacy of treatment with a catalytic antioxidant in a surrogate OP–exposure paradigm.[16]

Another attractive target to prevent OP-induced free radical damage is activation of the nuclear factor-like 2 (NRF2) transcription factor, which regulates the expression of antioxidant defenses. Although less is known about the efficacy of NRF2 activation in organophosphate exposure, treatment with the NRF2 activator sulforophane in a kindled-seizure model inhibited oxidative stress and protected neurons from free radical–mediated damage.[71]

Conclusions

There are multiple shared mechanisms by which redox imbalance and oxidative stress occur following seizures and OP exposures (Fig. 1). OP-induced oxidative stress, regardless of its source, can lead to neuronal injury. The use of catalytic antioxidants as neuroprotective agents may provide a therapeutic avenue for the treatment of acute and long-term neurological consequences of OP exposure. A deeper understanding of how OP-induced oxidative stress leads to neuronal injury may provide novel approaches for therapeutic interventions.

Acknowledgments

This work was supported by grants from the National Institutes of Health to M.P. (NIH R01NS39587, NIHRO1NS086423 and UO1NS083422) and J.N.P. (F31NS086405).

References

1. Jett DA. Finding new cures for neurological disorders: A possible fringe benefit of biodefense research? Science Translational Medicine. 2010; 2(23):23ps12–23ps12.
2. Jett DA, Yeung DT. The counteract research network: Basic mechanisms and practical applications. Proceedings of the American Thoracic Society. 2010; 7(4):254–256. [PubMed: 20601628]
3. Shih TM, Koviak TA, Capacio BR. Anticonvulsants for poisoning by the organophosphorus compound soman: Pharmacological mechanisms. Neurosci. Biobehav. Rev. 1991; 15(3):349–362. [PubMed: 1683477]
4. McDonough JH Jr. Clark TR, Sloan TW Jr, et al. Neural lesions in the rat and their relationship to eeg delta activity following seizures induced by the nerve agent soman. Neurotoxicology. 1998; 19(3):381–391. [PubMed: 9621344]
5. Brown MA, Brix KA. Review of health consequences from high-, intermediate- and low-level exposure to organophosphorus nerve agents. Journal of Applied Toxicology. 1998; 18(6):393–408. [PubMed: 9840747]
6. Silbergleit R, Lowenstein D, Durkalski V, et al. Rampart (rapid anticonvulsant medication prior to arrival trial): A double-blind randomized clinical trial of the efficacy of im midazolam versus iv lorazepam in the pre-hospital treatment of status epilepticus by paramedics. Epilepsia. 2011; 52(Suppl 8):45–47.
7. Wilhelm CM, Snider TH, Babin MC, et al. A comprehensive evaluation of the efficacy of leading oxime therapies in guinea pigs exposed to organophosphorus chemical warfare agents or pesticides. Toxicology and applied pharmacology. 2014; 281(3):254–265. [PubMed: 25448441]

8. International Programme on Chemical Safety. Organophosphorus pesticides. World Health Organization, Geneva Poisons Information Monograph G001. 1999. 1989
9. Turrens JF. Mitochondrial formation of reactive oxygen species. The Journal of Physiology. 2003; 552(Pt 2):335–344. [PubMed: 14561818]
10. Gardner PR, Fridovich I. Inactivation-reactivation of aconitase in escherichia coli. A sensitive measure of superoxide radical. Journal of Biological Chemistry. 1992; 267(13):8757–8763. [PubMed: 1315737]
11. McCord JM, Fridovich I. Superoxide dismutase: An enzymic function for erythrocuprein (hemocuprein). Journal of Biological Chemistry. 1969; 244(22):6049–6055. [PubMed: 5389100]
12. Mavelli I, Rigo A, Federico R, et al. Superoxide dismutase, glutathione peroxidase and catalase in developing rat brain. Biochemical Journal. 1982; 204(2):535–540. [PubMed: 7115348]
13. Lopert P, Patel M. Nicotinamide nucleotide transhydrogenase (nnt) links the substrate requirement in brain mitochondria for hydrogen peroxide removal to the thioredoxin/peroxiredoxin (trx/prx) system. The Journal of Biological Chemistry. 2014; 289(22):15611–15620. [PubMed: 24722990]
14. Kemp M, Go Y-M, Jones DP. Non-equilibrium thermodynamics of thiol/disulfide redox systems: A perspective on redox systems biology. Free radical biology & medicine. 2008; 44(6):921–937. [PubMed: 18155672]
15. Waldbaum S, Patel M. Mitochondrial dysfunction and oxidative stress: A contributing link to acquired epilepsy? Journal of bioenergetics and biomembranes. 2010; 42(6):449–455. [PubMed: 21132357]
16. Pearson JN, Rowley S, Liang LP, et al. Reactive oxygen species mediate cognitive deficits in experimental temporal lobe epilepsy. Neurobiology of Disease. 2015; 82:289–297. [PubMed: 26184893]
17. Liang LP, Ho YS, Patel M. Mitochondrial superoxide production in kainate-induced hippocampal damage. Neuroscience. 2000; 101(3):563–570. [PubMed: 11113305]
18. Liang LP, Patel M. Seizure-induced changes in mitochondrial redox status. Free Radical Biology and Medicine. 2006; 40(2):316–322. [PubMed: 16413413]
19. Ryan K, Liang LP, Rivard C, et al. Temporal and spatial increase of reactive nitrogen species in the kainate model of temporal lobe epilepsy. Neurobiology of Disease. 2014; 64:8–15. [PubMed: 24361554]
20. Rong Y, Doctrow SR, Tocco G, et al. Euk-134, a synthetic superoxide dismutase and catalase mimetic, prevents oxidative stress and attenuates kainate-induced neuropathology. Proceedings of the National Academy of Sciences. 1999; 96(17):9897–9902.
21. Liang LP, Waldbaum S, Rowley S, et al. Mitochondrial oxidative stress and epilepsy in sod2 deficient mice: Attenuation by a lipophilic metalloporphyrin. Neurobiology of Disease. 2012; 45(3):1068–1076. [PubMed: 22200564]
22. Glutekin F, Ozturk M, Akdogan M. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro). Arch. Toxicol. 2000; 74(9):533–538. [PubMed: 11131033]
23. Gupta J, Datta C, Sarkar A, et al. Effect of malathion on antioxidant defense system in human fetus – an in vitro study. Indian J. Exp. Biol. 1992; 30(4):352–354. [PubMed: 1459609]
24. Soltaninejad KMA. Current opinion on the science of organophosphate pesticides and toxic stress: A systematic review. Med. Sci. Monit. 2009; 15(3):RA75–90. [PubMed: 19247260]
25. Islam F, Tayyaba K, Hasan M. Organophosphate metasystox-induced increment of lipase activity and lipid peroxidation in cerebral hemisphere: Diminution of lipids in discrete areas of the rat brain. Acta Pharmacologica et Toxicologica. 1983; 53(2):121–124. [PubMed: 6624479]
26. Bagchi D, Bagchi M, Hassoun EA, et al. In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology and Applied Pharmacology. 1995; 104(1–3):129–140.
27. Gupta RC, Milatovic D, Zivin M, et al. Seizure-induced changes in energy metabolites and effects of n-tert-butyl-alpha-phenylnitrone (pnb) and vitamin e in rats. Pflugers Arch. Eur. J. Physiol. 2000; 44:R160–162.

28. Slotkin TA, Oliver CA, Seidler FJ. Critical periods for the role of oxidative stress in the developmental neurotoxicity of chlorpyrifos and terbutaline, alone or in combination. Developmental Brain Research. 2005; 157(2):172–180. [PubMed: 15963356]
29. Brocardo PS, Pandolfo P, Takahashi RN, et al. Antioxidant defenses and lipid peroxidation in the cerebral cortex and hippocampus following acute exposure to malathion and/or zinc chloride. Toxicology. 2005; 207(2):283–291. [PubMed: 15596258]
30. Kaur P, Radotra B, Minz RW, et al. Impaired mitochondrial energy metabolism and neuronal apoptotic cell death after chronic dichlorvos (op) exposure in rat brain. NeuroToxicology. 2007; 28(6):1208–1219. [PubMed: 17850875]
31. Slotkin TA, MacKillop EA, Ryde IT, et al. Screening for developmental neurotoxicity using pc12 cells: Comparisons of organophosphates with a carbamate, an organochlorine, and divalent nickel. Environmental Health Perspectives. 2007; 115(1):93–101. [PubMed: 17366826]
32. Tüzmen MN, Candan N, Kaya E. The evaluation of altered antioxidative defense mechanism and acetylcholinesterase activity in rat brain exposed to chlorpyrifos, deltamethrin, and their combination. Toxicology Mechanisms and Methods. 2007; 17(9):535–540. [PubMed: 20020880]
33. Brocardo PS, Assini F, Franco JL, et al. Zinc attenuates malathion-induced depressant-like behavior and confers neuroprotection in the rat brain. Toxicological Sciences. 2007; 97(1):140–148. [PubMed: 17327255]
34. Giordano G, Afsharinejad Z, Guizzetti M, et al. Organophosphorus insecticides chlorpyrifos and diazinon and oxidative stress in neuronal cells in a genetic model of glutathione deficiency. Toxicology and Applied Pharmacology. 2007; 219(2–3):181–189. [PubMed: 17084875]
35. Lukaszewicz-Hussain A. Subchronic intoxication with chlorfenvinphos, an organophosphate insecticide, affects rat brain antioxidative enzymes and glutathione level. Food and Chemical Toxicology. 2008; 46(1):82–86. [PubMed: 17706853]
36. Trevisan R, Uliano-Silva M, Pandolfo P, et al. Antioxidant and acetylcholinesterase response to repeated malathion exposure in rat cerebral cortex and hippocampus. Basic & Clinical Pharmacology & Toxicology. 2008; 102(4):365–369. [PubMed: 18341513]
37. Ranjbar A, Pasalar P, Abdollahi M. Induction of oxidative stress and acetylcholinesterase inhibition in organophosphorous pesticide manufacturing workers. Human & Experimental Toxicology. 2002; 21(4):179–182. [PubMed: 12099619]
38. Ranjbar A, Solhi H, Mashayekhi FJ, et al. Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. Environmental Toxicology and Pharmacology. 2005; 20(1):88–91. [PubMed: 21783573]
39. Shadnia S, Azizi E, Hosseini R, et al. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. Human & Experimental Toxicology. 2005; 24(9):439–445. [PubMed: 16235732]
40. Ryan K, Backos DS, Reigan P, et al. Post-translational oxidative modification and inactivation of mitochondrial complex i in epileptogenesis. The Journal of Neuroscience. 2012; 32(33):11250–11258. [PubMed: 22895709]
41. Muniz JF, McCauley L, Scherer J, et al. Biomarkers of oxidative stress and DNA damage in agricultural workers: A pilot study. Toxicology and Applied Pharmacology. 2008; 227(1):97–107. [PubMed: 18086483]
42. Kazi AI, Oommen A. Monocrotophos induced oxidative damage associates with severe acetylcholinesterase inhibition in rat brain. NeuroToxicology. 2012; 33(2):156–161. [PubMed: 22285544]
43. Dekundy A, Kaminski RM, Zielinska E, et al. Nmda antagonists exert distinct effects in experimental organophosphate or carbamate poisoning in mice. Toxicology and Applied Pharmacology. 2007; 219(2–3):114–121. [PubMed: 17157343]
44. McDonough JH Jr, Shih T-M. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology1. Neuroscience & Biobehavioral Reviews. 1997; 21(5):559–579. [PubMed: 9353792]
45. Patel M, Day BJ, Crapo JD, et al. Requirement for superoxide in excitotoxic cell death. Neuron. 1996; 16(2):345–355. [PubMed: 8789949]

46. Garthwaite J, Garthwaite G, Palmer RM, et al. Nmda receptor activation induces nitric oxide synthesis from arginine in rat brain slices. Eur. J. Pharamacol. 1989; 172(4-5):413–416.
47. Girouard H, Wang G, Gallo EF, et al. Nmda receptor activation increases free radical production through nitric oxide and nox2. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009; 29(8):2545–2552. [PubMed: 19244529]
48. Raza M, Blair RE, Sombati S, et al. Evidence that injury-induced changes in hippocampal neuronal calcium dynamics during epileptogenesis cause acquired epilepsy. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101(50):17522–17527. [PubMed: 15583136]
49. Deshpande LS, Carter DS, Blair RE, et al. Development of a prolonged calcium plateau in hippocampal neurons in rats surviving status epilepticus induced by the organophosphate diisopropylfluorophosphate. Toxicological Sciences. 2010; 116(2):623–631. [PubMed: 20498005]
50. Deshpande LS, Carter DS, Phillips KF, et al. Development of status epilepticus, sustained calcium elevations and neuronal injury in a rat survival model of lethal paraoxon intoxication. Neurotoxicology. 2014; 44:17–26. [PubMed: 24785379]
51. Bredt DS. Endogenous nitic oxide synthesis: Biological functions and pathophysiology. Free Rad. Res. 1999; 31(577-596)
52. Milatovic D, Gupta RC, Dekundy A, et al. Carbofuran-induced oxidative stress in slow and fast skeletal muscles: Prevention by memantine and atropine. Toxicology. 2005; 208(1):13–24. [PubMed: 15664429]
53. Zaja-Milatovic S, Gupta RC, Aschner M, et al. Protection of dfp-induced oxidative damage and neurodegeneration by antioxidants and nmda receptor antagonist. Toxicology and applied pharmacology. 2009; 240(2):124–131. [PubMed: 19615394]
54. Middlemore-Risher M-L, Adam B-L, Lambert NA, et al. Effects of chlorpyrifos and chlorpyrifos-oxon on the dynamics and movement of mitochondria in rat cortical neurons. The Journal of Pharmacology and Experimental Therapeutics. 2011; 339(2):341–349. [PubMed: 21799050]
55. Fukushima T, Hojo N, Isobe A, et al. Effects of organophosphorous compounds on fatty acid compositions and oxidative phosphorylation system in the brain of rats. Experimental and Toxicologic Pathology. 1997; 49(5):381–386. [PubMed: 9455686]
56. Gupta RC, Milatovic D, Dettbarn W-D. Depletion of energy metabolites following acetylcholinesterase inhibitor-induced status epilepticus: Protection by antioxidants. NeuroToxicology. 2001; 22(2):271–282. [PubMed: 11405258]
57. Gupta RC, Milatovic D, Dettbarn W-D. Nitric oxide modulates high-energy phosphates in brain regions of rats intoxicated with diisopropylphosphorofluoridate or carbofuran: Prevention by n-tert-butyl-alpha-phenylnitrone or vitamin e. Arch. Toxicol. 2001b; 75:346–356. [PubMed: 11570692]
58. Rowley S, Liang L-P, Fulton R, et al. Mitochondrial respiration deficits driven by reactive oxygen species in experimental temporal lobe epilepsy. Neurobiology of Disease. 2015; 75:151–158. [PubMed: 25600213]
59. Wani WY, Gudup S, Sunkaria A, et al. Protective efficacy of mitochondrial targeted antioxidant mitoq against dichlorvos induced oxidative stress and cell death in rat brain. Neuropharmacology. 2011; 61(8):1193–1201. [PubMed: 21784090]
60. Lee JE, Park JH, Shin IC, et al. Reactive oxygen species regulated mitochondria-mediated apoptosis in pc12 cells exposed to chlorpyrifos. Toxicology and Applied Pharmacology. 2012; 263(2):148–162. [PubMed: 22714038]
61. Binukumar BK, Gupta N, Sunkaria A, et al. Protective efficacy of coenzyme q10 against ddvp-induced cognitive impairments and neurodegeneration in rats. Neurotoxicity Research. 2011; 21(4):345–357. [PubMed: 22083459]
62. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cellular and Molecular Life Sciences CMLS. 61(2):192–208.
63. Sunkaria A, Wani WY, Sharma DR, et al. Dichlorvos-induced cell cycle arrest and DNA damage repair activation in primary rat microglial cells. Journal of Neuroscience Research. 2013; 91(3): 444–452. [PubMed: 23280485]

64. Pizzurro DM, Dao K, Costa LG. Astrocytes protect against diazinon- and diazoxon-induced inhibition of neurite outgrowth by regulating neuronal glutathione. Toxicology. 2014; 318:59–68. [PubMed: 24561003]

65. Jarrett SG, Liang L-P, Hellier JL, et al. Mitochondrial DNA damage and impaired base excision repair during epileptogenesis. Neurobiology of disease. 2008; 30(1):130–138. [PubMed: 18295498]

66. Wong-ekkabut J, Xu Z, Triampo W, et al. Effect of lipid peroxidation on the properties of lipid bilayers: A molecular dynamics study. Biophysical Journal. 2007; 93(12):4225–4236. [PubMed: 17766354]

67. Day BJ. Catalytic antioxidants: A radical approach to new therapeutics. Drug Discovery Today. 2004; 9(13):557–566. [PubMed: 15203091]

68. Day BJ, Crapo JD. A metalloporphyrin superoxide dismutase mimetic protects against paraquat-induced lung injuryin vivo. Toxicology and Applied Pharmacology. 1996; 140(1):94–100. [PubMed: 8806874]

69. Day BJ, Fridovich I, Crapo JD. Manganic porphyrins possess catalase activity and protect endothelial cells against hydrogen peroxide-mediated injury. Archives of Biochemistry and Biophysics. 1997; 347(2):256–262. [PubMed: 9367533]

70. Day BJ, Batinic-Haberle I, Crapo JD. Metalloporphyrins are potent inhibitors of lipid peroxidation. Free Radical Biology and Medicine. 1999; 26(5–6):730–736. [PubMed: 10218663]

71. Wang W, Wu Y, Zhang G, et al. Activation of nrf2-are signal pathway protects the brain from damage induced by epileptic seizure. Brain Research. 2014; 1544:54–61. [PubMed: 24333359]

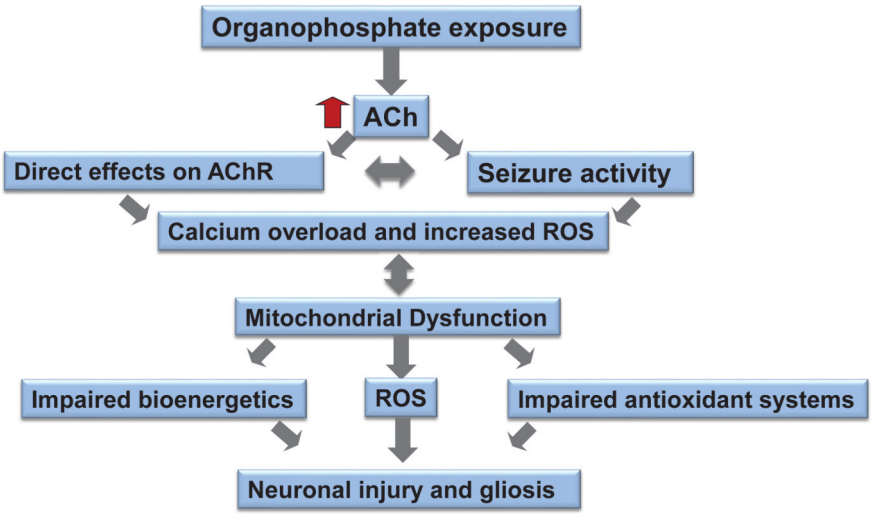

Figure 1.
Mechanisms by which redox imbalance and oxidative stress occur following seizures and OP exposures.