

Organophosphate-induced inhibition of acetylcholinesterase, oxidative stress and neuroinflammation

Yueqi Bai ^{1, †}, Luozheng Wang ^{2, *, †}

¹ Shanghai Shangde Experimental School, Shanghai, 201300, China

² School of Medical Science, Shandong University, Jinan, 250012, Shandong Province, China

* Corresponding author: 201900411086@mail.sdu.edu.cn

[†]These authors contributed equally.

Abstract. Organophosphate (OP) neurotoxicants exert their toxicity by inhibiting acetylcholinesterase. Overstimulation of cholinergic receptors can rapidly lead to neuronal damage, seizures, death, and long-term neurological damage in survivors. This review summarizes the mechanisms by which OP agents inhibit acetylcholinesterase action and lead to pathological acetylcholine overload *in vivo*, with attention to the effects of chronic and low-dose toxicity. Importantly, the massive accumulation of ROS during oxidative stress caused by OP agents are found to widely present in all toxic reactions. Moreover, OP agents can cause the release of pro-inflammatory cytokines from astrocytes, microglia, and increase the levels of prostaglandins and is prostaglandins, leading to neuroinflammation. A comprehensive understanding of the mechanisms of op-agents could help develop rational therapeutic approaches to treat toxicant exposure. However, current treatment for organophosphorus agent poisoning is relatively limited. Further research on the mechanisms of neurotoxicity is required to find ways to detoxify and treat organophosphorus agents.

Keywords: Organophosphate, acetylcholinesterase, oxidative stress, neuroinflammation.

1. Introduction

Organophosphates (also referred to as phosphate esters, or Ops, Fig.1) are a group of organic phosphorus compounds having the typical configuration of $O=P(OR)_3$, usually a central phosphate molecule with an alkyl or aromatic substituent [1]. OPs act via inhibition of acetylcholinesterase (AChE) in neural cells, and their inhibition of AChE leads to a pathological excess of acetylcholine in the bodies. However, their effects are not restricted to the acute phase, but also have long-term chronic effects [2]. Neurotransmitters such as acetylcholine are critical for brain development, and even low levels of exposure can have dramatic biological effects [14]. Because of their easy absorption through the digestive tract, respiratory tract and skin, it becomes an important threat for both intentional and unintentional poisoning [3, 4].

The main mechanism of OPs intoxication is through inhibition of AChE, leading to an excessive accumulation of acetylcholine (ACh) in the body and causing acute muscarinic manifestations; also, Oxidative stress from exposure to OP poisoning may be implicated in neuronal damage in the brain; its activation of astrocytes and microglia with increased prostaglandins and is prostaglandins may lead to neuroinflammation.

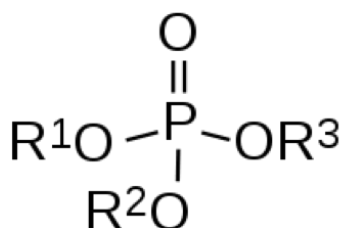


Figure 1. Structure of organophosphates.

The current means of treating OP poisoning with dephosphorylated binding agents and anticholinergic drugs such as atropine are expensive and ineffective, as well as having extremely

dangerous sequelae. Thus, it is necessary to inquire about alternative, more efficient treatments through the mechanisms of OP agent poisoning.

This review summarizes the AChE mechanisms of OP, the incidental oxidative stress and cytokines upregulated mechanisms to provide potential therapeutic means.

2. Acetylcholinesterase Inhibition

2.1. Acetylcholine

Acetylcholine is synthesized, stored and released by nerve endings and is a choline acetyltransferase synthesized from choline and acetyl coenzyme [5]. As one of important neurotransmitters, ACh plays an important role in sending signals at the neuromuscular junction and in the autonomic ganglia. Central cholinergic neurotransmission can significantly alter the excitability of neurons, alter presynaptic neurotransmitter release, and coordinate the fire of most neuronal groups [6].

In normal cholinergic transmission, the neurotransmitter ACh is liberated into the synapses in a respond to action potentials that reach the terminals of cholinergic neurons. ACh is used for preganglionic and postganglionic parasympathetic and sympathetic nervous system (sweat glands), central nervous system (CNS), and skeletal muscle motor end plates [7]. Cholinergic neurons which produce ACh are found throughout the brain, particularly in the brainstem, basal forebrain, striatum, and medial habenula. Cholinergic neurons play an important role in memory function [8].

2.2. Acetylcholine receptor

Acetylcholine affects the central and peripheral nervous system (PNS) via two different classes of receptors: muscarinic and nicotinic acetylcholine receptors (mAChRs and nAChRs). In brains, nAChRs are widely located at presynaptic and pre-terminal sites and are involved in a variety of functions, which include learning and memory, arousal, reward, movement control, and analgesia [9].

2.3. AChE and its Hydrolysis Activity

AChE is a key enzyme in the animal nervous system and, as a cholinergic enzyme of neurotransmitters, it can terminate neuronal transmission and signaling between synapses by rapidly degrading the neurotransmitter acetylcholine through hydrolytic activity. Organophosphates act as a neurotoxic agent that inhibits AChE in nerve cells and forces a toxic response.

The serine in the active site acts as a nucleophile to attack the carbon of the substrate to form a acyl-enzyme through a tetrahedral intermediate. The acyl enzyme is attacked by water molecule to break down to release acetic acid by the assisting of histidine group [10].

2.4. Inhibition of acetylcholinesterase

The toxicity of OPs mainly comes from irreversible inhibition of cholinesterase. OPs can inhibit the function of AChE by covalently binding to serine residues in the active site of AChE to block its active site. It inactivates AChE by irreversible phosphorylation of the esterase in the central nervous system.

When serine oxygen is activated by the electron movement of histidine, attached to a phosphorus. The enzyme forms a trigonal intermediate rather than a tetrahedral intermediate, and the tetrahedral intermediate forms a very stable phosphate ester when it breaks down. The phosphorylated enzyme is inactive and cannot hydrolyze the neurotransmitter [11]. Therefore, there would be too little AChE to degrade acetylcholine, leading to too much acetylcholine accumulated in the nervous system, which in turn would cause neurotoxicity.

Normally, the toxicity of OPs mainly comes from irreversible inhibition of cholinesterase. OPs can inhibit the function of AChE by blocking the active site of AChE. AChE can regulate the level of

ACh by catalysing the decomposition of ACh and producing choline and acetate at the synapse [12]. AChE has been found to be present in the nervous system, in neuromuscular junctions, and in red blood cells. When acetylcholine is not eliminated due to the inhibitory effect of AChE, ACh can be accumulated at synapses and at neuromuscular junctions, resulting in a range of symptoms, both nicotinic, muscarinic and central nervous system signs and symptoms (Table 1) [13].

Table 1. Organophosphate exposure signs and symptoms (Modified from [4])

Affected system	Potential signs and symptoms
Nicotinic	Tachycardia, weakness, dizziness, fascial contracture, hypertension
Muscarinic	Miosis, bradycardia, bronchospasm, bronchorrhea, emesis, defecation, urination, lacrimation, salivation, diaphoresis
Central nervous system	Confusion, seizures, anxiety, headaches

2.5. Chronic toxicity, Low-level exposure, and Cancer

Acetylcholine is extremely important in brain development, which leads to sequelae and long-term chronic effects of organophosphate exposure are present, even at low levels [14]. In addition to acute cholinergic toxicity (causing thousands of poisonings each year) delayed onset polyneuropathy is becoming increasingly frequent [15].

3. Oxidative stress

Oxidative stress is defined as a state of excess reactive oxygen species (ROS) production and/or the reduction in scavenging antioxidants, which results in pathophysiological changes that are similar to the general adaption syndrome of cellular stressors [16]. The common species of ROS are superoxide ($O_2^{\cdot-}$), hydroxyl ($\cdot OH$) radicals and peroxide ($ROO^{\cdot}$), and hydrogen peroxide (H_2O_2). In healthy states, the production of reactive oxygen species is usually quite modest and has an impressive signalling function [17]. Excessive ROS may rapidly be inactivated by the various antioxidants present in the organism. However, excessive production of reactive oxygen species and/or dysfunctional defense of antioxidants may lead to exposure of sensitive cellular components (DNA, proteins, lipids) to ROS-induced damage and result in cellular damage [18]. Three possible sources of ROS may cause oxidative stress after exposure to OPs, including excitotoxicity, mitochondrial dysfunction and inhibition of endogenous antioxidant activity.

3.1. Excitotoxicity

It has been suggested that the glutamate system may be at least partially responsible for the toxic effects of exposure [18,19]. Glutamate is one of the most abundant excitatory neurotransmitters in the central nervous system and is mainly inactivated in the normal brain through the "glutamate-Mon glutamine" cycle. Excessive accumulation of glutamate can cause excitatory neurotoxicity in neurons, so it is important to remove synthetic glutamate after it has exerted its neurotransmitter effect in order to maintain normal neuronal activity.

The OP reagent causes excessive accumulation of glutamate in the brain, which activates the NMDA receptor, which, when activated, increases ROS by synthesizing nitric oxide from the neuronal NO synthase (nNOS) linked to it [18]; at the same time, NMDA receptor activation induces ROS production through NOX₂ containing NADPH oxidase [20,21].

Importantly, the brain is particularly vulnerable to oxidative damage because it has a large lipid composition. The lipid composition, due to its high energy requirements, lacks a rich antioxidant defense system in comparison to other organs. As soon as oxidative damage prevails over endogenous antioxidant defense systems and reparative processes, neuronal death and gliosis may occur [22]. Evidence for this is that in animal experiments it was shown that the addition of sub-nanomolar concentrations of Ngb1 when exposed to H_2O_2 prevented ROS accumulation, reduced antioxidant

enzymes, and decreased the expression of apoptotic proteins in astrocytes [23]. In addition, neuronal loss in the hippocampal portal and CA3 regions of rats treated with pilocarpine was attenuated by treatment with a broad-spectrum catalytic antioxidant with SOD, catalase and lipid peroxidation inhibitory properties [24].



Figure 2. Mechanisms of ROS production of Glutamate system

3.2. Mitochondrial dysfunction

Damaged mitochondria are also one of the most important sources of excess ROS. Studies have shown that acute exposure to OPs and chronic low-level exposure to OPs also increase calcium levels in mitochondria, impair a variety of electron transport chain (ETC) complexes, reduce mitochondrial SOD (superoxide dismutase) and induce apoptosis. This is supported by the fact that (a) chronically low levels of exposure to Dichlorvos in rats were proven to lead to depression of mitochondrial complex I and cytochrome oxidase in their brains, and thus to the production of ROS [25]. Excessive ROS formation causes destruction of cellular antioxidant defenses with cytochrome c (cyt c) release from mitochondria into the cytoplasm, leading to apoptosis. (b) Meanwhile, mitochondrial complexes I, II, III and IV of the ETC are suppressed by acutely exposed OP or a substitute for OP. Propionol exposure (DFP, an organophosphate) has been demonstrated to dramatically drain ATP levels in the amygdala and hippocampus. Seizures generate a high ATP requirement and the consequence of this, when combined with an inhibition of all facets of the ETC, is an impairment of energy production and an overproduction of ROS, particularly O_2^- and H_2O_2 [26].

3.3. Inhibition of endogenous antioxidant activity

In research on OP toxicity in the brain, experiments have found that some endogenous antioxidant systems, such as Superoxide dismutase (SOD), are reduced following OP exposure. Studies have shown that acute and sub-acute exposure is adequate to reduce the activity of glutathione peroxidase, glutathione reductase, GSH, SOD and catalase [27]. These key antioxidant enzymes are essential to prevent oxidant-induced damage to cellular macromolecules, i.e., carbohydrates, lipids, proteins, and nucleic acids [28]. In the presence of excessive ROS production, i.e., Weakening of the enzymatic activity of crucial antioxidants during seizures may assist in OP-induced oxidative stress, which would likely lead to neuronal damage [29].

4. Neuroinflammation

Organophosphates may cause neuroinflammation in terms of neurotoxicity (Fig. 3). Inhibition of acetylcholinesterase (AChE) by OPs can cause cholinergic toxicity, that leads to damage in neurons through the release of cytokines derived from activated microglia and astrocytes. The release of prostaglandins/isoprostanoids and damage in neurons because of increased excitotoxicity are another two results of OP intoxication. On the one hand, exposure to OPs can activate astrocytes and microglia. Activated astrocytes and microglia can release several chemokines and proinflammatory cytokines, which can cause neuroinflammation. On the other hand, OPs can lead to the increase of prostaglandins and isoprostanoids. Prostaglandins and isoprostanoids are closely related to inflammation, and can promote the process of neuroinflammation in central nervous system (CNS) [30].

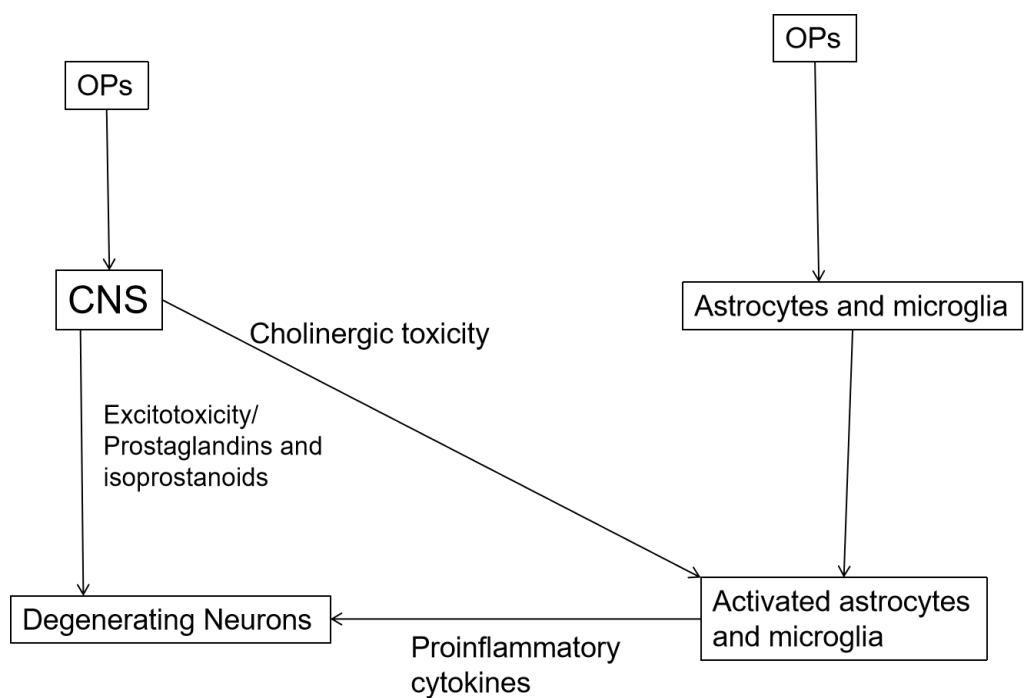


Figure 3. Inflammatory response after exposure to OPs. Modified from [30]

4.1. Cytokine upregulation induced by OP toxicity

One of main characteristics of inflammatory reaction is the release of cytokines (Fig. 4). Organophosphates can activate microglia and astrocytes, which can produce more cytokines. Activated microglia can produce more IL-1 α , β , et al., while activated astrocytes can produce more proinflammatory cytokines. Cytokines derived from microglia can activate astrocytes. OPs can lead to massive GFAP over expression, which can cause astrogliosis.

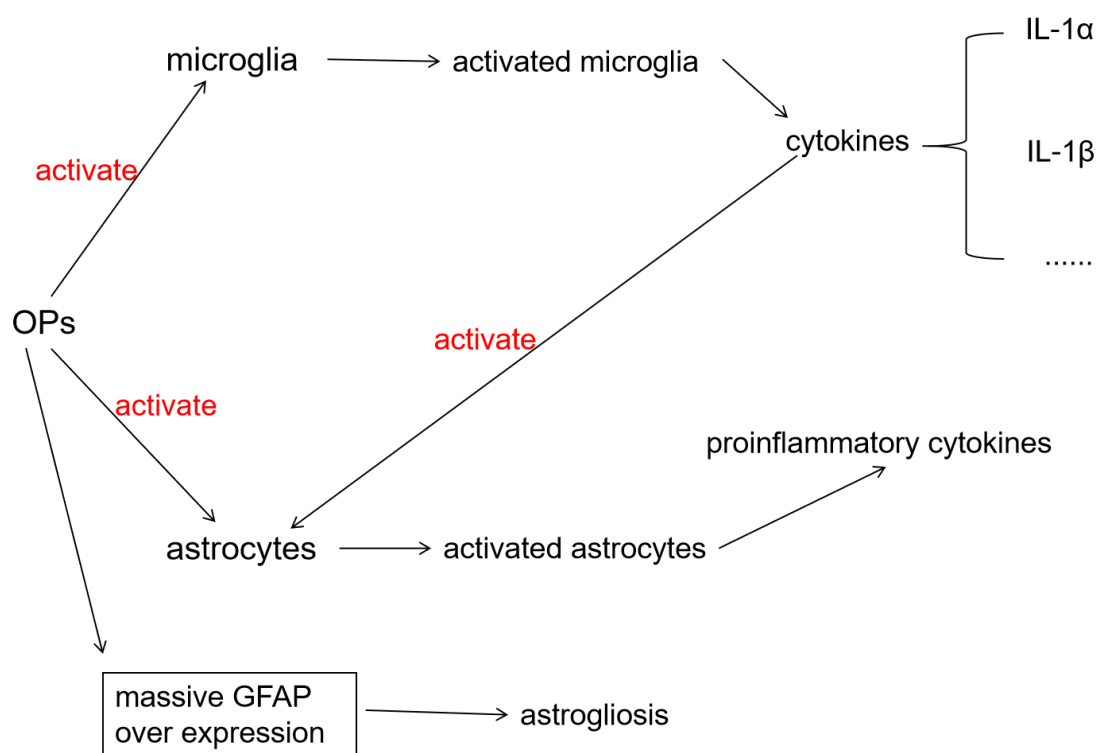


Figure 4. Schematic of upregulating cytokines by OP intoxication.

4.1.1. The activation of microglia and astrocytes

OPs can cause activation of microglia and astrocytes, that leads to changes of their structures and changes in expression of cytokines. Activated microglia can release chemokines and proinflammatory cytokines (Fig. 4) [28]. Studies have revealed that OP intoxication could cause a severe neuroinflammatory response which activates microglia and astrocytes. Exposure to OPs may change the microstructure of major cortical connections. Furthermore, neuroinflammation may be associated with morphological and connectivity shifts in neurons and glia, which may be one of the reasons why microglia can be activated after exposure to OPs [31].

The increase of activated microglial cells was found in several places in brain, including the lateral septum, cingulate cortex, claustrum, amygdala, thalamus and hippocampus. The changes of activated microglia cells were in structure, such as larger cytoplasm and thicker processes. And activated microglia can produce IL-1 α and β , while non-activated microglia can only express IL-1 β [36]. Changes of these pro-inflammatory factors in turn promote the process of neuroinflammation. There are several lines of evidence suggested that IL-1 α is a top mediator of neuroinflammation. After cellular injury, release of IL-1 α , or its transferring to the cell membrane can lead to signal transduction through IL-1R via a paracrine and autocrine way. IL-1R signaling causes expression and release of cytokines and chemokines, such as IL-1 α and β , which can recruit inflammatory cells to the sites of damage and promote the process of inflammation [32].

Cytokines derived from microglia can activate astrocytes [33]. Activated astrocytes can produce proinflammatory cytokines, which is associated with the promotion of neuroinflammation [28]. In addition, OPs, such as Somen, can lead to massive GFAP over-expression in brains. This can cause long-term astrogliosis [29]. The increasing number of astrocytes can also promote the development of neuroinflammation.

4.1.2. The upregulation of cytokines

OPs may lead to greatly increased expression of select pro-inflammatory mediators in the cortex and hippocampus. Exposure to diisopropylfluorophosphate (DFP), for example, led to an increased expression of CCL2 and TNF- α in cortex and hippocampus [34]. In another research, the levels of IL-1 β , IL-6 and TNF- α increased within 2h following the exposure of Sarin, one kind of organophosphates [35]. Activated microglia can synthesize IL-1 α and β , while dystrophic microglia can only express IL-1 β [36]. And Diethyldithiophosphate (DEDTP) can significantly increase TNF- α , IL-1 β , and PDGF mRNA [37]. Moreover, high expression level of CXCL1 and MIP-1 α , another two cytokines can be induced by Soman in hippocampus, the piriform cortex and thalamus [39].

However, in another research, after Phosphorus flame retardant (PFR) exposure, the production of anti-tumor cytokines IFN-2 α and TNF- β , which can promote inflammation, were reduced [38]. However, not all the pro-inflammation cytokines were reduced. Importantly, there are both pro-inflammatory and anti-inflammatory effects in CNS after exposure to OPs. A potential explanation is that there are negative feedback mechanisms trying to maintain homeostasis.

Pro-inflammatory cytokines can lead to the degeneration of neurons, which promotes the process of neuroinflammation. And microglial activation in response to neuronal damage is really fast [30]. Thus, it could be seen as a positive-feedback loop mechanism that leads to severe inflammation.

4.2. Prostaglandins and isoprostaglandins implicated in OP poisoning

Prostaglandin E2 (PGE2) is a physiologically active lipid. Through the catalysis of the cyclooxygenases (COX-1 and COX-2) and PGE synthases, PGE2 can be synthesized from arachidonic acid (AA) in vivo [40]. PGE2 is intimately associated with inflammation (Fig. 5). Isoprostanooids, including F2-isoprostanes (F2-IsoPs) and neuron-specific F4-neuroprostanes (F4-NeuroPs), are synthesized by peroxidation of AA [41]. Isoprostanooids can help to increase the ability of neutrophil to adhere to endothelial cells and may promote inflammatory response by increasing neutrophil activity [42].



Figure 5. Prostaglandin E2 (PGE2) can be biosynthesized from AA through the catalysis of the cyclooxygenases (COX-1 and COX-2) and PGE synthases.

4.2.1. Prostaglandin E2

After exposure to intoxicating doses of Sarin, decreased level of PGE2 receptor transcripts can be found in the brain [43]. The levels of PGE2 protein are increased in the hippocampus and cortex of rats [35].

OPs increase COX-2 expression. For example, after exposure to Soman, great increases in the amount of COX-2 immunoreactive cells were seen in the piriform cortex and amygdala. The increases of COX-2 can lead to seizure which is a mental symptom. And COX-2 expression had positive relationship with seizure Intensity [44]. The effect of COX-2 induction in hippocampal neurons is very significant after DFP exposure [45]. However, OPs don't alter the expression of COX-1. There are no apparent changes by Soman in the expression of COX-1. In addition, Soman enhanced COX-2 expression in neurons *in vivo* but not in microglia [44].

4.2.2. Isoprostanooids

Acute OP intoxication induces the production of isoprostanooids. A study found remarkable increases of F2-IsoPs and F4-NeuroPs in rats upon an acute exposure to DFP. DFP-induced increases in F2-IsoPs and F4-NeuroPs were along with dendritic degeneration in pyramidal neurons in the CA1 hippocampal area [46]. This phenomenon may be the result of AChE inhibition [41].

5. Conclusions

OPs cause neurotoxicity through multiple mechanisms that vary depending on the mode of exposure. The most common effects of OPs agents are the inhibition of AChE, which may lead to low-dose and chronic effects. Besides, the significant role of excess ROS in the overall mechanisms and the neurotoxicity induced have been found to play an important role when OPs inhibit AChE. However, substantial questions persist regarding the functional relationships between oxidative stress, neuroinflammation, and seizures, as well as the mechanisms through which OPs triggered such processes and their roles. Further research is required to addressing these questions to potentially provide more effective therapeutic tools for the treatment of OPs caused neurotoxicity.

References

- [1] A. Greaves, R. Letcher, D. Chen, D. McGoldrick, L. Gauthier and S. Backus, Retrospective analysis of organophosphate flame retardants in herring gull eggs and relation to the aquatic food web in the Laurentian Great Lakes of North America, *Environmental Research* 150 (2016) 255 - 263.
- [2] G. Velmurugan, T. Ramprasath, K. Swaminathan, G. Mithieux, et al., Gut microbial degradation of organophosphate insecticides-induces glucose intolerance via gluconeogenesis, *Genome Biology* 18 (2017).
- [3] S. Narayan, Z. Liew, K. Paul, P. Lee, J. Sinsheimer, J. Bronstein and B. Ritz, Household organophosphorus pesticide use and Parkinson's disease, *International Journal of Epidemiology* 42 (2013) 1476 - 1485.
- [4] L. Razwiedani and P. Rautenbach, Epidemiology of Organophosphate Poisoning in the Tshwane District of South Africa, *Environmental Health Insights* 11 (2017) 117863021769414.
- [5] I. Muramatsu, T. Masuoka, J. Uwada, H. Yoshiki, T. Yazama, K. Lee, K. Sada, M. Nishio, T. Ishibashi and T. Taniguchi, A New Aspect of Cholinergic Transmission in the Central Nervous System, *Nicotinic Acetylcholine Receptor Signaling in Neuroprotection* (2018) 45 - 58.
- [6] M. Picciotto, M. Higley and Y. Mineur, Acetylcholine as a Neuromodulator: Cholinergic Signaling Shapes Nervous System Function and Behavior, *Neuron* 76 (2012) 116 - 129.

- [7] P. Taylor, Anticholinesterase Agents, in: L.L. Brunton, R. Hilal-Dandan, and B.C. Knollmann (Eds), Goodman & Gilman's: The Pharmacological Basis of Therapeutics, McGraw-Hill Education, New York, 2017, chapter 10.
- [8] J. Wess, Molecular Biology of Muscarinic Acetylcholine Receptors, *Critical Reviews™ in Neurobiology* 10 (1996) 69 - 99.
- [9] M. Zoli, S. Pucci, A. Vilella and C. Gotti, Neuronal and Extraneuronal Nicotinic Acetylcholine Receptors, *Current Neuropharmacology* 16 (2018) 338 - 349.
- [10] D. Nachmansohn and I. Wilson, The Enzymic Hydrolysis and Synthesis of Acetylcholine, *Advances in Enzymology - and Related Areas of Molecular Biology* (2006) 259 - 339. M. Colovic, D. Krstic, T. Lazarevic-Pasti, A. Bondzic and V. Vasic, Acetylcholinesterase Inhibitors: Pharmacology and Toxicology, *Current Neuropharmacology* 11 (2013) 315 - 335.
- [11] M. Shah, W. Ren, J. Irudayaraj, A. Sajini, M. Ali and B. Ahmad, Colorimetric Detection of Organophosphate Pesticides Based on Acetylcholinesterase and Cysteamine Capped Gold Nanoparticles as Nanozyme, *Sensors* 21 (2021) 8050.
- [12] K.J. Richardson, J.L. Schwinck, and M.V. Robinson, Organophosphate poisoning, *Nurse Pract.* 46 (2021) 18 - 21.
- [13] C.D. Klaassen, Casarett and Doull's toxicology: the basic science of poisons, eighth ed., McGraw-Hill, New York, 2013.
- [14] T. Cole, J. Fisher, T. Burbacher, L. Costa and C. Furlong, Neurobehavioral assessment of mice following repeated postnatal exposure to chlorpyrifos-oxon, *Neurotoxicology and Teratology* 34 (2012) 311 - 322.
- [15] H. Sies, C. Berndt and D. Jones, Oxidative Stress, *Annual Review of Biochemistry* 86 (2017) 715 - 748.
- [16] J. Garthwaite, G. Garthwaite, R. Palmer and S. Moncada, NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices, *Eur. J. Pharmacol.: Molecular Pharmacology* 172 (1989) 413 - 416.
- [17] H. Girouard, G. Wang, E. Gallo, J. Anrather, P. Zhou, V. Pickel and C. Iadecola, NMDA Receptor Activation Increases Free Radical Production through Nitric Oxide and NOX2, *Journal of Neuroscience* 29 (2009) 2545 - 2552.
- [18] D. Bredt, Endogenous nitric oxide synthesis: Biological functions and pathophysiology, *Free Radical Research* 31 (1999) 577 - 596.
- [19] M. Patel, B. Day, J. Crapo, I. Fridovich and J. McNamara, Requirement for Superoxide in Excitotoxic Cell Death, *Neuron* 16 (1996) 345 - 355.
- [20] J. Pearson and M. Patel, The role of oxidative stress in organophosphate and nerve agent toxicity, *Annals of the New York Academy of Sciences* 1378 (2016) 17 - 24.
- [21] V. Kumar and K. Gill, Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: A review, *NeuroToxicology* 41 (2014) 154 - 166.
- [22] F. Amri, I. Ghouili, M. Amri, A. Carrier and O. Masmoudi-Kouki, Neuroglobin protects astroglial cells from hydrogen peroxide-induced oxidative stress and apoptotic cell death, *Journal of Neurochemistry* 140 (2016) 151 - 169.
- [23] J. Pearson, S. Rowley, L. Liang, A. White, B. Day and M. Patel, Reactive oxygen species mediate cognitive deficits in experimental temporal lobe epilepsy, *Neurobiology of Disease* 82 (2015) 289 - 297.
- [24] P. Kaur, B. Radotra, R. Minz and K. Gill, Impaired mitochondrial energy metabolism and neuronal apoptotic cell death after chronic dichlorvos (OP) exposure in rat brain, *Neurotoxicology* 28 (2007) 1208 - 1219.
- [25] S. Rowley and M. Patel, Mitochondrial involvement and oxidative stress in temporal lobe epilepsy, *Free Radical Biology and Medicine* 62 (2013) 121-131.;
- [26] E. Kurutas, the importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state, *Nutrition Journal* 15 (2015).
- [27] E. N. Benveniste, Cytokine actions in the central nervous system, *Cytokine Growth Factor Rev.* 9 (1998) 259 - 75.
- [28] J. M. Collombet, E. Four, D. Bernabe, C. Masqueliez, et al., Soman poisoning increases neural progenitor proliferation and induces long-term glial activation in mouse brain, *Toxicology*. 208 (2005) 319 - 34.

- [29] C.N. Banks and P. J. Lein, A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation, *Neurotoxicology*. 33 (2012) 575 - 84.
- [30] B. B. Koo, L.T. Michalovicz, S. Calderazzo, K.A. Kelly, et al., Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness, *Brain Behav Immun*. 67 (2018) 42 - 46.
- [31] A. Malik and T.D. Kanneganti, Function and regulation of IL-1alpha in inflammatory diseases and cancer, *Immunol Rev*. 281 (2018) 124 - 137.
- [32] B. Kaminska, A. Gozdz, M. Zawadzka, A. Ellert-Miklaszewska, et al., MAPK signal transduction underlying brain inflammation and gliosis as therapeutic target, *Anat Rec (Hoboken)*. 292 (2009) 1902 - 13.
- [33] A. R. Locker, L. T. Michalovicz, K.A. Kelly, J.V. Miller, et al., Corticosterone primes the neuroinflammatory response to Gulf War Illness-relevant organophosphates independently of acetylcholinesterase inhibition, *J Neurochem*. 142 (2017) 444 - 455.
- [34] S. Chapman, T. Kadar, and E. Gilat, Seizure duration following sarin exposure affects neuro-inflammatory markers in the rat brain, *Neurotoxicology*. 27 (2006) 277 - 83.
- [35] E. A. Johnson and R.K. Kan, The acute phase response and soman-induced status epilepticus: temporal, regional and cellular changes in rat brain cytokine concentrations, *J Neuroinflammation*. 7 (2010) 40.
- [36] B. J. Proskocil, A. C. G. Grodzki, D.B. Jacoby, P. J. Lein, et al., Organophosphorus Pesticides Induce Cytokine Release from Differentiated Human THP1 Cells, *Am J Respir Cell Mol Biol*. 61 (2019) 620 - 630.
- [37] X. Li, N. Li, K. Rao, Q. Huang, et al., In Vitro Immunotoxicity of Organophosphate Flame Retardants in Human THP-1-Derived Macrophages, *Environ Sci Technol*. 54 (2020) 8900 - 8908.
- [38] E.A. Johnson, T. L. Dao, M.A. Guignet, C.E. Geddes, et al., Increased expression of the chemokines CXCL1 and MIP-1alpha by resident brain cells precedes neutrophil infiltration in the brain following prolonged soman-induced status epilepticus in rats, *J Neuroinflammation*. 8 (2011) 41.
- [39] K. Tsuge, T. Inazumi, A. Shimamoto, and Y. Sugimoto, Molecular mechanisms underlying prostaglandin E2-exacerbated inflammation and immune diseases, *Int Immunol*. 31 (2019) 597-606.
- [40] D. Milatovic, R.C. Gupta, and M. Aschner, Anticholinesterase toxicity and oxidative stress, *ScientificWorldJournal*. 6 (2006) 295 - 310.
- [41] S. Zahler and B.F. Becker, Indirect enhancement of neutrophil activity and adhesion to cultured human umbilical vein endothelial cells by isoprostanes (iPF2alpha-III and iPE2-III), *Prostaglandins Other Lipid Mediat*. 57 (1999) 319 - 31.
- [42] T. V. Damodaran, S. T. Greenfield, A.G. Patel, H. K. Dressman, et al., Toxicogenomic studies of the rat brain at an early time point following acute sarin exposure, *Neurochem Res*. 31 (2006) 367 - 81.
- [43] M. Angoa-Perez, C.W. Kreipke, D. M. Thomas, K.E. Van Shura, et al., Soman increases neuronal COX-2 levels: possible link between seizures and protracted neuronal damage, *Neurotoxicology*. 31 (2010) 738 - 46.
- [44] A. Rojas, T. Ganesh, W. Wang, J. Wang, et al., A rat model of organophosphate-induced status epilepticus and the beneficial effects of EP2 receptor inhibition, *Neurobiol Dis*. 133 (2020) 104399.
- [45] S. Zaja-Milatovic, R.C. Gupta, M. Aschner, and D. Milatovic, Protection of DFP-induced oxidative damage and neurodegeneration by antioxidants and NMDA receptor antagonist, *Toxicol Appl Pharmacol*. 240 (2009) 124 - 31.