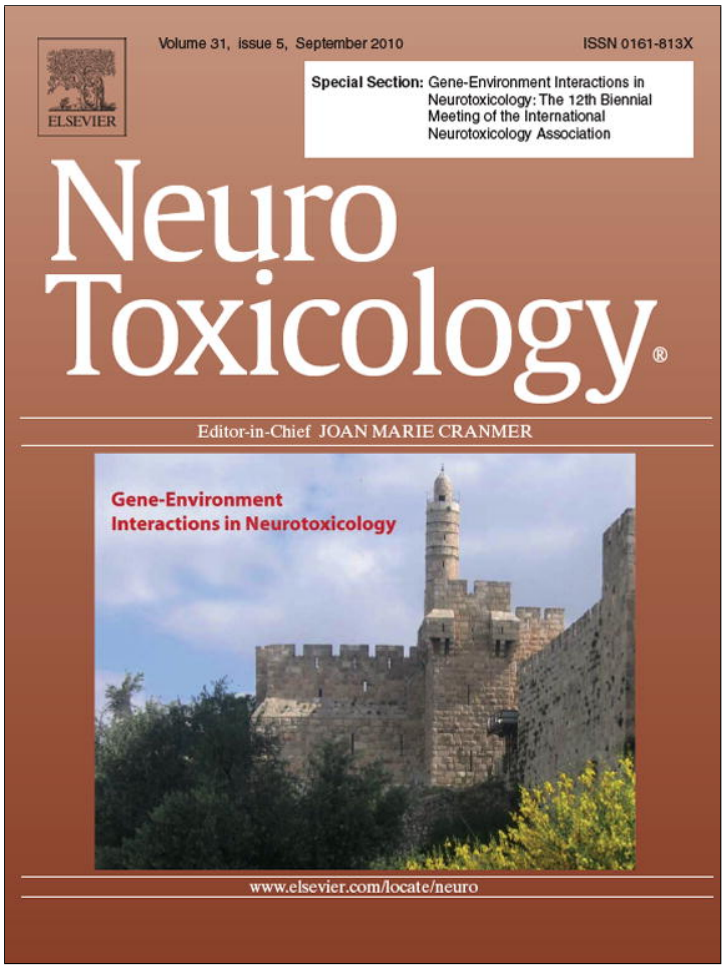

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Peaceful use of disastrous neurotoxicants

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ABSTRACT

The increasing exposure to environmental neurotoxicants in the last decades caused serious health problems in the world population. Some of the neurotoxic agents are being used in agriculture and household such as insecticides and rodenticides and others are of natural origin like snake and scorpion venoms. Additional group of harmful substances is the chemical warfare agents including nerve and blistering agents that are known for their disastrous effects on neuronal tissues. The present paper presents a combination of epidemiological/clinical and molecular approaches for investigating the effect of certain groups of neurotoxicants on a variety of pathologies.

The work of Finkelstein and coworkers describes epidemiological and clinical studies on acute and chronic organophosphate (OP)-induced neurotoxicity in certain populations in Israel. They mainly investigated the neurotoxic effects of low-level long-term exposure to OP in agricultural areas but also dealt with acute exposures as well. A molecular approach to OP mechanism of neuronal injury was described by Milatovic and coworkers. They demonstrated OP-induced oxidative injury in pyramidal neurons in the CA1 hippocampal area and its suppression by antioxidants. Lecht and coworkers described the novel snake venom angioneurins as important mediators of the physiological cross-talk between the cardiovascular and nervous systems. They also showed that under certain conditions these angioneurins may induce pathologies such as tumor development or disruption of the vascular barrier function during envenomation. Additional mechanistic/therapeutic approach was presented by Brodsky, Rosengarten, Proscura, Shapira and Wormser. They developed a novel anti-inflammatory peptide that reduced skin irritation induced by heat and sulfur mustard (SM) stimuli. Since SM causes neuropsychiatric symptoms and alterations in neurological functions this peptide may serve as a potential treatment of neuronal injuries caused by environmental neurotoxicants.

These reviews highlight different aspects of neurotoxicity, addressing epidemiology and mechanisms of toxicity; and identifying novel potential therapies.

IsIs there a consistent cognitive and neurobehavioral pattern of toxicity following acute, delayed or chronic exposures to organophosphates?

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Introduction

Interest in the neurotoxic effects of organophosphates (OPs) in humans began in 1932 when Longe and von Krueger noted the self-toxic effects of the vapors of OP compounds which they just had synthesized (Besser and Guttman, 1995). Since then, intense research has being carried out. Most of the studies have dealt with acute OP neurotoxicity, probably because the potential use of OP warfare remains a serious concern. Thus, worldwide interest in OP has been aimed at the acute clinical manifestations following short-term high-dose exposure.

Most of the research on agricultural OP poisoning, an additional worldwide problem (World Resources Institute, 1994–1995) has also been dedicated to the acute clinical picture and to the life-saving procedures. The more subtle central nervous system (CNS) effects, first and foremost the impairment in higher cognitive functions, have received less attention (Steenland et al., 1994; Steenland, 1996).

The first study dedicated to the possible linkage between acute OP poisoning and chronic neurobehavioral changes was carried out in 1950. In this clinical study, two patient groups (affective bipolar and schizophrenic patients) and a healthy control group were injected daily with diisopropylfluorophosphate (DFP) for one week. Depressive mood was observed in the bipolar patients and in the normal controls, while behavioral deterioration and lengthy exacerbation of psychosis were observed in the schizophrenic patients (Rowntree et al., 1950). Similar schizophrenic and depressive symptomatology was described a decade later in patients who had been chronically exposed to OP for several years (Gershon and Shaw, 1961), but a detailed epidemiological study (Stoller et al., 1965) challenged these earlier observations. Later studies which employed neurobehavioral test batteries, showed a sub-clinical decrease in the cognitive performance of individuals who had previously had a single (Rosenstock et al., 1991) or repeated episodes (Savage et al., 1988) of acute OP poisoning.

The delayed CNS effects of acute OP poisoning have also been described scantily.

Neurophysiologic outcomes were detectable months or even years following recovery from acute OP poisoning (Savage et al., 1988; Yokoyama et al., 1998).

Recently, a dose–response association was found between low-dose inhalation exposure to the OP warfare agents sarin and cyclosarin during the 1991 Gulf War and delayed impaired neurobehavioral functioning as well as subtle CNS pathology as revealed by a magnetic resonance imaging (MRI) study (Heaton et al., 2007; Proctor et al., 2006).

The effects of low-level long-term exposures have attracted more attention, due to the increasing concern on the health hazards of the widespread use of pesticides in agricultural communities. However, studies designed to examine the possible neurobehavioral effects of chronic exposures are relatively sparse. In most of the studies, there are considerable uncertainties regarding exposures. Evidently, human exposures include other classes of pesticides such as fungicides and fumigants. Furthermore, most of the exposures are to mixtures of chemicals. Neurobehavioral test batteries showed several effects of chronic OP exposure in adult working populations: deficits in measures of motor speed and coordination, sustained attention, and information processing speed (Reidy et al., 1992; Steenland et al., 1994). Neurobehavioral changes have also been reported in farm workers in Florida (Kamel et al., 2003): farm work was associated with poor performance on Digit Span, tapping, and Santa Ana Tests. In cotton pesticides applicators in Egypt presumed to have heavy exposures, a broad range of signs was observed: visual motor slowliness, reduced verbal abstraction, attention deficit and memory impairment (Farahat et al., 2003).

Association of long-term exposure and worse performance in neuropsychological functions was shown in a cross-sectional survey of greenhouse workers in high-exposure conditions in Spain. The study was performed, using a wide array of tasks to test neuropsychological functioning and emotional status. The variable "years working with pesticides" was found to be a measure of cumulative exposure for risk of worsened perceptive function performance, visuomotor praxis and integrative task performance time with no relation to plasma ChE activity as a measure of recent exposure (Roldán-Tapia et al., 2005).

However, the studies of subjects with long-term low-level OP exposures show overall inconsistent findings, and only few studies have reported the association between pesticide exposure and neurological endpoints (Rothlein et al., 2006). Evidently, OP may produce a spectrum of clinical manifestations in human, ranging from convulsion and cardiac arrhythmia, coma and death (at the high-end acute exposures) to sub-clinical neurotoxicity with implicit cognitive deficits and mild neurobehavioral impairments (at the low-end chronic exposures). But the clinical and sub-clinical signs have not painted a comprehensive and consistent clinical picture, and no well-defined anatomical common denominator has yet been defined. Furthermore, there is no clear distinction among the different clinical forms of OP poisoning following different rates and duration of exposures and the clinical data are sometimes conflicting.

Several studies in Israel on acetyl cholinesterase (AChE) inhibitors (excluding carbamates) have tried to resolve this conflicting evidence and to find a common denominator for the gamut of clinical manifestations. Several approaches have been undertaken to address this question: (a) quantitative histochemical analysis of AChE activity in fatal cases of acute OP poisoning; (b) a clinical study on severe acute consecutive cases of OP poisoning; (c) studies on the neurobehavioral effects of low-level short-term OP exposure in Israel; (d) an observational clinical study on the delayed sequelae of acute OP poisoning; (e) epidemiological field studies on the effects of low-level long-term OP exposure in the 1980s; (f) current re-examination of the same cohorts by the same as well as novel methods.

The principal clinical findings of all the studies on the neurobehavioral effects of OP poisonings which have been carried out in Israel between 1970 and 2010 are summarized herein and may well provide the Israeli perspective.

(a) Quantitative histochemical analysis of acetyl cholinesterase (AChE) activity in the human brain in severe acute cases of OP poisoning

For nearly four decades, OP pesticides have been the most commonly used pest-control agents in cotton and orchard cultivation in Israel (Richter et al., 1992a,b; Bar-Ilan et al., 2000; Bar-Ilan and Malman, 2007). Most reported instances of acute OP poisoning in Israel have been due to agricultural pesticides (Finkelstein et al., 1988a,b; Weissmann-Brenner et al., 2002). Quantitative histochemical analysis of brain AChE activity was

performed in stained sections of parathion-poisoned brain of a 19-year-old woman and compared to an age- and sex-matched control (Finkelstein et al., 1989). In this study, the pattern of AChE inhibition in the brain was shown to be regionally selective. The most significant decreases in AChE activity were observed in the cerebellum, thalamic nuclei and the cerebral cortex – first and foremost in the frontal and temporal lobes. In several cortical gyri of the frontal lobes, AChE activity was decreased by 56–86% in the parathion-poisoned case, as compared to the control brain. In several cortical gyri of the temporal lobes, AChE activity was decreased by 33–60% in the parathion-poisoned case, as compared to the control brain. (Finkelstein et al., 1988c). The cholinergic dysfunction could be well correlated with the histochemical changes in AChE activity, since cortical AChE may serve as a reasonably good index of cholinergic activity (Mesulam et al., 1986).

(b) Clinical studies on severe acute cases of OP poisoning in Israel

Both experimental and clinical data for OP exposure cases admitted over a five-year period to the intensive care units in the Israeli hospitals have been integrated in a study (Finkelstein et al., 1989), as a basis for a widely accepted standard protocol of emergency treatment of OP poisonings (Finkelstein, 1992). During the period of this study, 856 consecutive consultations for patients exposed to OP pesticides were requested from the Israel National Poison Control Center which serves all the Israeli population. Fifty-three patients were included in the study. Criteria of inclusion were severe OP poisoning necessitating artificial ventilation, intensive care monitoring and antidotal treatment. Thirty-two patients (60%) had major CNS involvement, seizures or coma. Seven patients (13%) died during hospitalization. All the fatalities in that consecutive series were the result of severe hypoxemic respiratory failure associated with cardiac arrhythmia, specifically polymorphic ventricular tachycardia. The total incidence of the potentially fatal ventricular arrhythmias did not correlate with the degree of the CNS involvement or AChE inhibition. However, these full-blown arrhythmias correlated with the severity of respiratory failure with consequent myocardial anoxia (Israel National Poison Control Center, 1987).

The experience accumulated by the pediatric emergency department and intensive care unit in the main medical center in the Negev in Southern Israel has been analyzed in several reports (Leibson and Lifshitz, 2008; Lifshitz et al., 1994, 1999; Sofer et al., 1989). The analysis of these cases pointed out different clinical presentations of pediatric toxicity compared to the classic clinical presentation of OP toxicity in adults. The most common presentation in these children was obtunded state of consciousness, stupor or coma alongside hypotonic muscle weakness and dyspnea. Lack of history of exposure and absence of classical signs do not exclude the possibility of OP poisoning. These factors only stress the importance of a high index of clinical suspicion required in the differential diagnosis of acute OP poisoning in children.

(c) Studies on the neurobehavioral effects of low-level short-term OP exposure in Israel

The consequence of short-term exposures to OP on neurotoxic outcomes has been previously studied (Richter, 1987, 1993; Richter et al., 1984, 1986). Major findings from these studies attest to OP effects on clinical parameters and neurobehavioral tests, both in occupational and community sentinel groups in Israel. The occupational groups studied included aerial spray pilots, ground-crews, and field workers – crop inspectors, irrigation workers and repair mechanics. The studies addressed levels of urinary metabolites for the detection of low-level exposures, short-term illness and neuropsychological decrements. Mean peak-season scores in the OP-exposed workers and residents were slightly poorer (in all cases $<10\%$) than mean post-season scores for the following tests of performance: Reaction Time, Santa Ana Test, Digit Symbol Test, Benton Visual Retention Test, Digit Span (Scaled Score), Trail Making Tests A and B, and Block Design Test (Number and Scale Score). Peak-season scores were substantially worse ($>10\%$ difference) for tests of mood state and symptoms, e.g., POMS fatigue–inertia, anger–hostility and tension–anxiety, as well as depression. Furthermore symptoms and metabolite levels were lower in groups with work exposures to fields with reduced pesticide applications (Meng et al., 1996).

(d) Observations on the delayed sequelae of acute OP poisoning in Israel

A psychiatric disorder, manifested as a prominent delayed effect of severe acute OP poisoning, was evidenced in several patients examined a multi-hospital study. In this study, 5 out of 53 cases (9.4%) presented severe psychiatric dysfunction after the resolution of the acute respiratory failure. The clinical presentation resembled regression psychosis. The psychiatric syndrome resolved in all the patients within 3–5 weeks. The incidence of this psychiatric syndrome in patients above the age of 15 was 5/31 (16.1%) (Finkelstein et al., 1989).

A preliminary study of the delayed long-term effects of a single acute OP poisoning on cognitive behavior in seventeen 6–12-year-old Bedouin nomadic children was conducted in the Negev (Kofman et al., 2006). A neuropsychological assessment was performed in these children, who had been hospitalized in infancy following exposure to OP pesticides. The findings were compared with findings in children exposed to other toxicants, and in age- and sex-matched non-exposed children. All the exposed children attended regular schools, but their cognitive assessment pointed to impairment in the verbal learning and motor inhibition tasks. In the verbal learning test, the difference between the exposed children and controls was found in the rate of learning, but not in delayed recall and recognition. The increased gain in the recognition phase in exposed children suggests that they showed impaired retrieval only in the acquisition phase, but not after the delay, suggesting that both encoding and consolidation processes remained intact. Subtle behavioral changes were evident in the tests which assessed these children's inhibitory control. They had greater difficulties controlling their motor behavior vs. matched controls. These findings suggested that subtle behavioral changes may occur even if they go undetected in school.

(e) Epidemiological field studies on the neurobehavioral effects of low-level long-term OP exposure in Israel

A higher incidence of neurological symptoms, fatigue, and psychiatric disturbances was described in the Negev in garden pest control, orchard and cotton workers with long-term exposure, although no association was found with ChE activity levels (Ilani et al., 1988).

Several studies which were carried out in 1977–1987 (Israel National Poison Control Center, 1987; Richter, 1993) assessed the neurobehavioral effects of long-term low-level OP exposures in orchard residents of several kibbutzim in the Hula Valley in Northern Israel. Three cohorts were examined: agricultural workers, workers in the kibbutzim but not in agriculture and residents working elsewhere with no occupational or drift exposures. These studies demonstrated dose–response gradients linking symptoms of illness with the measured levels of OP metabolites in residents and workers. Neurobehavioral tests in adults showed subtle short-term reversible changes in measures of mood, mental and motor performance. Short-term memory, attention and time to reaction performance were more impaired in agricultural workers than in other residents of the kibbutzim

(Richter, 1993). Dose–response relationships have been observed between these effects and the measured levels of OP metabolites in urine samples of children in the same kibbutzim (Richter et al., 1992a).

(f) Epidemiological field studies on the long-term neurobehavioral effects of low-level long-term OP exposure – current re-examination of the 1980s cohorts

We are currently revisiting the same cohorts which were examined in the 1980s to assess long-term effects of these past and subsequent exposures to OP pesticides. We recently generated a unique database of these original cohorts of workers and residents for future follow-up. Several tests from the WHO Core Test Battery (Richter, 1987), cognitive tests and a subjective symptoms questionnaire – POMS (Profile of Mood States) are being employed. A follow-up over a 20-year period on OP exposures in human cohorts has yet to be reported. The preliminary observations of the current cognitive tests in 60 individuals of the revisited cohorts show mild signs of impairment in the performance of executive tasks in the sub-group of individuals with high cumulative occupational exposure. This sub-group performed significantly lower than the sub-group of individuals with no occupational exposure. The difference was evident first and foremost in Trail Making Tests A and B for screening, attention and graphomotor ability, and Digit Symbol Test for eye-hand coordination in new learning processes and picture completion for visual perception of objects.

Additionally, we collected information on hyperactivity or attention deficit disorder and unexamined anecdotal reports of regional associations with OP exposure. Our present preliminary observations in Hula Valley children are based on the primary findings of the review questionnaires: Twenty-four out of the sixty (40%) respondents in all the original cohorts reported that at least one of their children had been diagnosed with attention deficit/hyperactivity disorder (ADD/ADHD). The number of children diagnosed with ADD/ADHD was 51 out of 204 (25%) children in these 60 families. A few have suggested (Fogelman et al., 2003; Kaufmann et al., 2009) that ADD/ADHD affects as many as 5–7% of the children in Israel, but no relevant data exist in the Israel Central Bureau of Statistics. ADD/ADHD affects 5–9% of the children in the USA (Pastor and Reuben, 2008). In a comparison of our preliminary data to the preexisting data: the difference between these two proportions is highly significant ($p < 0.0001$).

Discussion

The anatomical selectivity of OP-induced cholinergic effects which were shown in our studies is compatible with the clinical correlates of poisoning, involving impairment of emotional response and decline in higher cortical functions.

Inhibited AChE activity in the frontal lobes is compatible with the clinical frontal subdominant sign of impaired reading comprehension (Bowers et al., 1964), the frontal dominant signs of expressive language defects (Grob and Harvey, 1953; Namba et al., 1971) with intermittent pauses and perseveration (Bowers et al., 1964).

These frontal signs are the first to appear in acute OP poisoning (Koeller and Klawans, 1979; Finkelstein, 1989b). The bilateral frontal signs of psychomotor slowing (Luria, 1970) and reduced cognitive efficiency (Koeller and Klawans, 1979; Richter et al., 1992a) may appear in both acute and chronic OP poisonings.

The neurobehavioral changes described in acute, delayed and chronic forms of OP poisoning (Finkelstein et al., 1989; Richter et al., 1992a; Ilani et al., 1988; Kofman et al., 2006) may reflect the remarkable AChE inhibition observed in the mesial temporal lobe. Dysfunction of this area plays an important role in acute confusional states (Mesulam, 1985), mood (Metcalf and Holmes, 1969) and memory disturbances which are observed in both acute (Finkelstein et al., 1989) and chronic poisonings (Ilani, 1988; Kofman et al., 2006). Amnesic word finding difficulty, dominant temporal lobe impairment (Luria and Karasseva, 1968) is a clinical correlate of remarkable AChE inhibition in the dominant temporal lobe (Finkelstein et al., 1988a).

The current preliminary findings in individuals with low-level long-term occupational exposure to OP provide support to this regional effect on the frontal and temporal lobes. Indeed, reduced performance in Trail Making Test B reflects frontal lobe dysfunction (Reitan, 1955), as was corroborated by recent MRI/MRS correlative studies (Kochunov et al., 2010). The reduced performance of the same individuals in Digit Symbol Test indicates impairment of a frontal executive function involving the associative pathways among the frontal, posterior parietal and occipital lobes. Digit symbol is unique, being the only Wechsler subset that necessitates on-the-spot learning of an unfamiliar task (Gregory, 2007). The efficient performance requires the ability to rapidly produce distinctive verbal codes to represent each of the symbols in memory – the learning theory (Estez, 1974). Digit symbol is one of the sensitive sub-tests to the effects of organic brain impairment (Donders et al., 2001).

Overall, the prominent cognitive and emotional impairments are compatible with frontal and temporal lobe dysfunctions which are manifested more remarkably in the executive functions of the dominant cerebral hemisphere. The clinical findings point to consistent endpoints which may have a common anatomical denominator in acute high level exposure as well as in chronic low-level exposure to OP.

However, OP neurotoxicity is mediated by several mechanisms. OP-induced impairment of cognitive functions was shown to be probably caused by subsequent desensitization and internalization of cholinergic receptors as a reaction of OP-exposed organisms on hyper-stimulation of cholinergic receptors, especially in parts of the brain with a high density of cholinergic synapses such as the hippocampus (McDonald et al., 1988). This means that a decrease in the number of cholinergic receptors in the hippocampus following low-level exposure to OP without significant AChE inhibition could cause memory impairments. Although sometimes insignificant, some extent of AChE inhibition is caused even by low-level chronic exposure to OP. The levels of chronic exposures may vary over time and may be related to the seasons of agricultural activity. Therefore the receptors may be up- or down-regulated.

Complaints of weakness and tingling in hands and feet, together with minor changes in nerve conduction, suggest a differential diagnosis: these clinical symptoms and signs may derive from central nervous system or, alternatively, from peripheral nervous system (PNS) OP-induced effects. The PNS effect may be exerted independently from AChE activity; and is probably mediated by a neurotoxic esterase-type activity depending on which OP is the substrate.

Prody et al. (1989) reported the amplification of the gene coding for AChE activity in selected Israeli farmers with known heavy exposures to OP; the clinical implications of this are not clear.

A dose–response association was found between low-dose exposure to sarin and cyclosarin inhalation during the 1991 Gulf War and impaired neurobehavioral functioning as well as subtle CNS pathology as revealed by MRI study (Heaton et al., 2007; Proctor et al., 2006). It is noteworthy that functional impairments were detected even in people who initially developed only mild or no signs of sarin or cyclosarin toxicity.

A significant, clinically manifested AChE inhibition in the CNS, leading to the neuronal degeneration of some brain regions including the hippocampus is associated with spatial learning and memory impairments, often sub-clinical. This observation corre-

sponds with reports on neurological and neurophysiologic outcomes detectable months or even years following recovery from acute OP poisoning (Savage et al., 1988; Yokoyama et al., 1998).

The delayed effects of acute OP poisoning children (Kofman et al., 2006) included impairment in the verbal learning and motor inhibition tasks, with difficulties restraining and controlling the motor behavior. These findings point to disinhibition and release phenomena due to frontal lobe dysfunction.

Chronic exposure to OP compounds can also result in specific long-term cognitive deficits even when signs and symptoms of excessive cholinergic activity are not present (Prendergast et al., 1998). Anticholinesterase compounds may alter behavior functions even after small sub-toxic doses. Results describing the signs and symptoms in OP-exposed humans unclose some long-term health effects, including behavioral effects of repeated sub-clinical exposures to OP compounds (Wesseling et al., 2002). It was shown that psychological symptoms are probably more common than usually recognized and may persist in more subtle forms for much longer than physical symptoms (Sidell and Hurst, 1977). The data obtained in our studies correspond to the epidemiological studies elsewhere showing alterations in cognitive functions, impaired memory, and concentrations in humans after chronic low-dosage occupational exposure to OP insecticides (Parron et al., 1996; Stephens et al., 1995).

Based on the clinical observations and the cognitive findings in the Hula Valley cohorts as well as the other studies mentioned above, we suggest the same anatomical common denominator for acute (mild form), delayed and chronic OP neurotoxicity. The anatomical selectivity of OP-induced cholinergic effects is compatible with the clinical correlates of poisoning, involving impairment of emotional response and decline in higher cortical functions. Although regulatory and compensatory mechanisms exist, the underlying neuroanatomical deficits seem to be closely related to the clinical manifestations. If this is indeed the case, the different symptoms observed after the different types of exposure to OP could be first and foremost a reflection of the brain regions preferentially involved. However, the clinical picture of acute severe OP poisoning is different. This clinical difference derived from the predominant anoxia of both brain and myocardium with the consequent cardiac arrhythmias and diffuse anoxic brain damage (Finkelstein et al., 1988a).

Our preliminary finding of high ADD/ADHD prevalence in children residing in an agricultural region raises concern, as current theory suggests that a genetic factor is primarily responsible for the pathogenesis of ADHD (Banaschewski et al., 2010). There may be environmental-susceptibility interactions mediating the emergence of ADHD in populations with low-level endemic exposure to OP. Therefore, the significance of this finding should be addressed by additional tests, such as paroxonase-1 (PON-1) which might reflect the genetic susceptibility of children to the effects of OP long-term low-level exposures. In fact, not all OPs are hydrolyzed by PON-1 and furthermore, studies of the polymorphisms show different activities in different OPs used as substrate. The importance of the issue is magnified by the lack of contemporary data and therefore simultaneous measurement of urinary OP metabolites, characterizing the OP substances to which the examined individuals are exposed with single-nucleotide polymorphisms (SNP) in PON-1 is most likely to provide meaningful data on the relationship between exposures to different OPs, their metabolism and cognitive/physiological effects.

A recent study examined the association between urinary concentrations of six dialkyl phosphate metabolites of OPs and ADHD in children 8–15 years of age (Bouchard et al., 2010). These children were representative of the general US population, based on cross-sectional data from the National Health and Nutrition Examination Survey (2000–2004). Using a structured interview with a parent to ascertain ADHD diagnostic status, 119 out of 1139 children met the DSM-IV diagnostic criteria for ADHD (American Psychiatric Association, 1994). Children with higher urinary dialkyl phosphate concentrations were more likely to be diagnosed as having ADHD. There was a 55–72% increase in the odds of ADHD for a 10-fold increase in dimethyl alkylphosphate (DMAP) concentration, depending on the criteria used for case identification. For the most-commonly detected metabolite, dimethyl thiophosphate, children with levels higher than the median detectable concentrations had twice the odds of ADHD, compared with children with undetectable levels. These ADHD rates in OP-exposed children are similar to our preliminary findings. However, in this study the urine samples were collected in both agricultural and non-agricultural populations, and only once. For a comparison, our ongoing study is exclusively aimed at children in agricultural areas and the urine samples are being collected at different times, both in the spraying season and off-season.

The findings in the study of the Negev children (Kofman et al., 2006) suggested that subtle behavioral changes may occur, even if they go undetected in school. The outcomes of Statue test which was employed in this study were compatible with previous evidence, relating exposure to methyl parathion to a higher incidence of parental report impulsivity and conduct disorder (Ruckart et al., 2004). Impaired motor inhibition (Barkley, 1997) is one of the hallmarks of attention deficit hyperactivity disorder (ADHD), and indeed, the Statue test is included in the "attention" battery of the NEPSY (Korkman et al., 2001). These findings overall, may significantly contribute to the existing knowledge on the deleterious late cognitive effects of prolonged everyday long-term low-level exposure to environmental OP pesticides.

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Suppression of oxidative injury and neurodegeneration in cholinergic toxicity

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Introduction

Exposure to anticholinesterase agents, organophosphates (OPs) and carbamates (CMs) in the form of insecticides and chemical warfare agents affects a sizeable portion of the world's population. Presently, more than 100 different OPs are used as insecticides worldwide (Kwong, 2002). The advantage of the lower environmental stability and faster clearance of OPs compared with organochlorine pesticides as well as their high effectiveness against multiple insect species is accompanied by the disadvantage of high mammalian toxicity (Lotti, 2001). The widespread use and easy accessibility to these compounds result in a huge number of

intoxications and several hundred thousand fatalities annually (Gunnell and Eddleston, 2003).

Pharmacologically, OPs and carbamates are acetylcholinesterase (AChE) inhibitors and their acute symptoms are attributed to accumulation of acetylcholine (ACh), thus exhibiting the signs of cholinergic hyperactivity. Depending upon the degree of AChE inhibition, the severity of poisoning can vary from mild (mild dyspnea, blurred vision and glandular hypersecretion) to severe (severe dyspnea, skeletal muscle fasciculations, convulsions and unconsciousness) cases, and eventually death ensues from respiratory failure (Goldfrank et al., 1982; Weinbroum, 2004). However, anticholinesterases have long-term pathophysiological effects that have yet to be well characterized, making rational prophylaxis and treatment for these effects problematic. Long-term neurological impairments following anticholinesterase exposures are also associated with non-cholinergic mechanisms including oxidative injury and excitotoxicity. Therefore, control of oxidative stress and excitotoxicity, better understanding of the mechanisms of non-cholinergic mediated activities and pathways that protect or promote neuronal survival are essential for development of efficacious treatments and preventive therapies associated with OP exposures.

Oxidative stress and neurodegeneration

Results from our study with diisopropylphosphorofluoridate (DFP), a model compound for OP insecticides or nerve agents corroborate the findings, suggesting that the non-cholinergic system(s) is recruited at an early stage of the OP poisoning (Lallement et al., 1991; Wade et al., 1987). OP-induced seizures are thought to result in activation of glutamatergic neurons in the pyriform cortex (Wade et al., 1987) and cornu ammonis (CA) region of the hippocampus (Lallement et al., 1991) followed by activation of *N*-methyl-D-aspartate (NMDA) receptors in the CA1 region. Furthermore, excessive amounts of glutamate are associated with intense transient influx of Ca^{2+}, leading to mitochondrial structural and/or functional impairments, activation of the permeability transition pores in the inner mitochondrial membrane, cytochrome *c* release, depletion of ATP, stimulation of enzymes, including proteases, phospholipase A_2, and NOS and simultaneous formation of ROS and oxidative stress (Heinemann et al., 2002; Cadenas and Davies, 2000; Patel, 2002; Nicholls et al., 2003; Lafon-Cazal et al., 1993; Farooqui et al., 2001). Oxidative stress and modulations of Ca^{2+}, glutamate and NMDA receptors compromise cell viability, ensuing in neurodegeneration. The result from our study demonstrated that a single injection of DFP with an acute dose of 1.25 mg/kg, s.c. produced toxic signs in rats, including salivation, tremors, fasciculations within 15–20 min. Signs of maximal intensity, such as severe muscle fasciculations, seizures, and convulsions developed within 30 min and lasted for about 2–3 h before tapering off. By 24 h, animals were free of toxic signs. Following DFP exposure, brain AChE activity was reduced to $10.12 \pm 1.12\%$, $10.05 \pm 1.96\%$ and $16.51 \pm 1.27\%$ compared to control (569.12 ± 26.80 mol/h/g of tissue) at 30 min, 1 h, 2 h and 6 h, respectively. However, the result from our study also demonstrated that DFP-induced reversible oxidative damage to cerebral neuronal membranes accompanied with depletion of ATP (Zaja-Milatovic et al., 2009). Oxidative injury was quantified by measuring isoprostanes (F_2-IsoPs), chemically stable oxidative damage products of arachidonic acid (Morrow et al., 1990; Milatovic and Aschner, 2009) and neuroprostanes (F_4-NeuroPs, oxidative damage to docosahexaenoic acid – highly concentrated in neuronal membranes) that provide unique insight into oxidative damage occurring in neurons. Both biomarkers of oxidative damage showed transient increase that reached maximum at the time of the most intensive seizure activity (i.e., 1 h after DFP, 1.25 mg/kg, s.c.) in rat and returned toward basal levels by 6 h (Fig. 1). Elevated levels of these *in vivo* markers of oxidative damage are in agreement with our previous findings from the models of kainic acid-induced excitotoxicity and activated innate immunity (Milatovic et al., 2008, 2003) and indicate that DFP injection leads to profound cerebral and neuronal oxidative damage in mice.

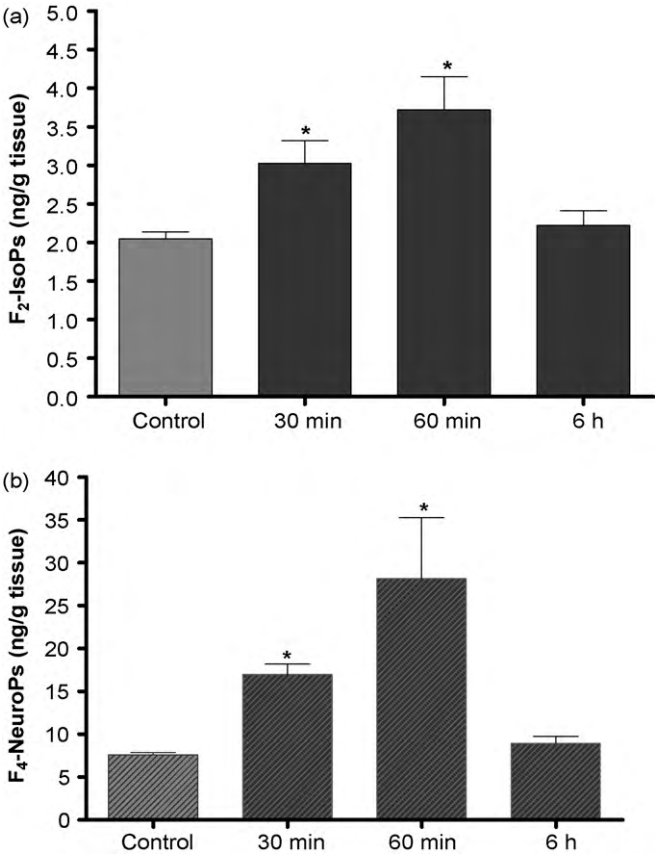

Fig. 1. Cerebral concentrations of F_2-IsoPs (a) and F_4-NeuroPs (b) following DFP (1.25 mg/kg, s.c.) exposure in rats. Values represent mean ± SEM ($n = 4$–6). *One-way ANOVA had $p < 0.001$ with Bonferroni's multiple comparison tests showed significant difference ($p < 0.05$) for vehicle injected control vs. DFP treatment.

Next, we investigated morphological correlates of DFP injections, determining the structural integrity of the hippocampal (CA1) dendritic system, the neuronal compartment most sensitive to both age-related and disease-related degeneration (Uylings and de Brabander, 2002). Using Golgi impregnation and Neurolucida-assisted morphometry, our results show that oxidative damage is accompanied with reduction in both dendrite length and dendritic spine density in hippocampal CA1 pyramidal neurons 1 h post-DFP injection (Fig. 2).

An additional goal of this study was to determine whether suppression of lipid peroxidation prevents neurodegeneration of pyramidal neurons in the CA1 hippocampal area in the model of DFP-induced neurotoxicity. Therefore, the efficacy of antioxidants [vitamin E and synthetic spin trapping agent, *N*-*tert*-butyl-α-phenylnitrone (PBN)] and the NMDA receptor antagonist, memantine, was evaluated. Antioxidants play an important role in preventing many human diseases, including but not limited to cancer, atherosclerosis, stroke, rheumatoid arthritis and neurodegeneration (Fang et al., 2002). Vitamin E is one of the most potent and important antioxidants, acting as a chain-breaking antioxidant and radical scavenger, thus protecting cells from peroxidation of polyunsaturated fatty acids in phospholipids and consequent membrane degeneration (van Acker et al., 1993). PBN is widely used to trap ROS in a variety of physical, chemical and biological conditions. Memantine exerts various pharmacological effects and

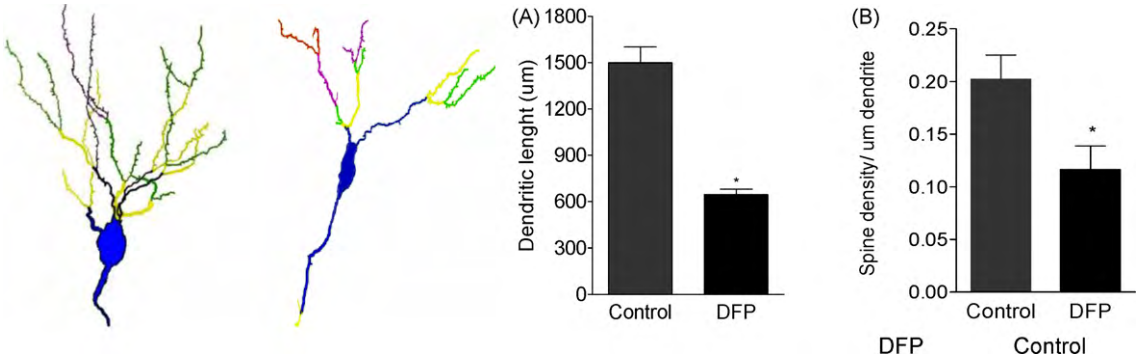

Fig. 2. Morphology and quantitative determination of dendritic length (a) and spine density (b) of hippocampal pyramidal neurons from CA1 sector of rats treated with saline (control) or DFP (1.25 mg/kg, s.c.) and sacrificed 1 h after the treatment. Four to six Golgi-impregnated dorsal hippocampal CA1 neurons were selected and spines counted by using Neurolucida system. *Significant difference between control and DFP-treated rats ($p < 0.05$). Treatment with DFP induced degeneration of hippocampal dendritic system, decrease in total length of dendrite and spine density of hippocampal pyramidal neurons. Tracing and counting are done using a Neurolucida system at 100× under oil immersion (MicroBrightField, VT). Colors indicate degree of dendritic branching (blue = 1°, yellow = 2°, green = 3°, magenta = 4°, orange = 5°). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

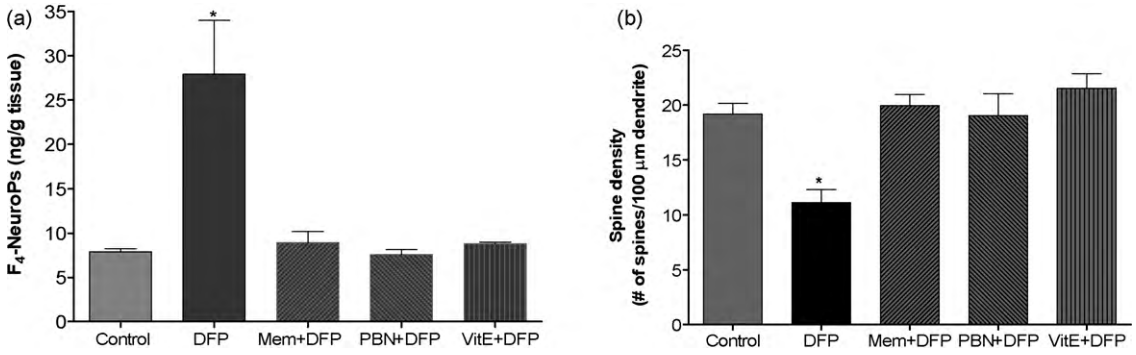

Fig. 3. Cerebral F_4-NeuroPs concentrations (a) and spine density of pyramidal neurons from CA1 hippocampal area (b) of rats following saline (control) or DFP (1.25 mg/kg, s.c.) exposed rats with or without pretreatment with memantine (MEM, 18 mg/kg, s.c.), *N-tert*-butyl-α-phenylnitrone (PBN, 200 mg/kg, i.p.) or vitamin E (Vit E, 100 mg/kg, i.p./day for 3 days). Brains from rats exposed to DFP were collected 1 h post injections. Values of F_4-NeuroPs and spine density represent mean ± SEM (n = 4–6 animals and more than 12 neurons). *One-way ANOVA had $p < 0.001$ with Bonferroni's multiple comparison tests significant ($p < 0.05$) for DFP vs. control, Mem + DFP, PBN + DFP or Vit E + DFP treatment.

is clinically used for the treatment of Alzheimer's disease, Parkinson's disease and spasticity (Ozsuer et al., 2005; Lipton, 2005). Results of our study confirmed the protective effects of these antioxidants and memantine, and showed that all agents fully suppressed DFP-induced increases in cerebral and neuronal markers of oxidative damage, F_2-IsoPs and F_4-neuroPs, respectively. In addition to attenuating DFP-induced increased production of markers of NO/NOS and depletion of ATP, all tested agents fully protected hippocampal CA1 pyramidal neurons from dendritic degeneration (Fig. 3). Therefore, suppression of lipid peroxidation, most likely mediated by scavenging ROS, and the parallel reduction in neuronal damage following the increase in antioxidants and memantine strongly supports oxidative stress mechanisms as causal mediators of DFP-induced seizures and neurodegeneration.

Conclusion

We have explored mechanisms associated with OP-induced neurotoxicity by probing their effects on oxidative stress and associated dendritic degeneration of pyramidal neurons in the CA1 hippocampal area. We have also investigated different pathways to attenuate biomarkers of oxidative damage associated with anticholinesterase exposure and the extent to which such attenuation is accompanied by rescue from neurodegeneration. Results from our studies suggest that vitamin E, PBN and memantine efficiently suppress oxidative injury. Future studies should be directed at deciphering the mechanisms of protection, addressing the ability of these agents to attenuate OP neurotoxicity via radical scavenging, AChE inhibition and/or glutamate antagonism. Additional studies should also investigate not only the prophylactic, but also therapeutic effects of these neuroprotectants. Successful identification of safe and effective neuroprotectants that suppress non-cholinergic activities associated with anticholinesterase exposure will provide new pharmacological modalities to protect and treat both the acute and delayed effects of nerve agent poisoning.

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Snake venom angioneurins: toxic or therapeutic growth factors?☆

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[☆]This study is part of the PhD thesis of SL to be submitted to The Hebrew University of Jerusalem.

Vipera palestinae venom toxicity

Snake bite is a serious medical problem in particular in Asia, Africa and often encountered in the Middle East countries including Israel. The main symptoms following these accidences include hemorrhagic activity expressed by edema, alterations in the coagulation system, myotoxicity expressed by massive muscle fibers collagen desheeting and myonecrosis, cardiotoxicity often expressed by strong hypotension and arrthymic pathologies, neurotoxicity expressed by flaccid paralysis and depression of central autonomic vasoregulatory mechanism and respiratory paralysis (Thwin and Gopalakrishnakone, 1998). Serotherapy is currently the only available therapy for treating snake envenomation. The common side effects associated with its use and the poor neutralization of the local or systemic effects of the venom (Weinstein et al., 2009) are calling for modern studies to evaluate the full proteomic composition and pathophysiological activity as well as development of more effective therapeutic strategies to deal with snake venom envenomation (Calvete et al., 2009). In Israel, the *Vipera xanthina palestinae* bites are among the most common reasons for human and veterinary envenomations (Aroch and Harrus, 1999). For this purpose the Israeli Ministry of Health is producing a *Vipera palestinae* antivenom containing polyclonal antibodies toward neurotoxins and hemorrhagins and based on formaldehyde detoxification of the venom (Moroz, 1998). *Vipera palestinae* venom HPLC fractions and MALDI-TOF proteomic analysis indicated the presence of complex mixtures of pharmacologicaly active molecules including (Fig. 4): (i) neurotoxins: 2% neurotoxic phospholipase A_2 (PLA_2) (Krizaj et al., 1996); (ii) myotoxins: 2% myotoxic PLA_2; (iii) hemorrhagins: 65% zink metallo proteinase, 9% serine proteinase, 10% C-type lectine-like disintegrin, 6% dimeric disintegrin, 1% cystein rich disintegrin, <1% short disintegrin (Kisiel et al., 2004) similar to the snake venomics composition of other north African viper venoms (Bazaa et al., 2005). In addition to these components, we also detected about 2% of the venom content as growth factors such as vascular endothelial growth factor (VEGF) (Brown et al., 2007) and nerve growth factor (NGF) (Lecht et al., 2008) known to induce vascular permeability (Kostiza et al., 1995; Weis and Cheresh, 2005).

Vipera palestinae venom-derived growth factors with therapeutic implications

In addition to the toxic effects, VEGF and NGF growth factors are also characterized by beneficial therapeutic effects such as: angiogenesis (Lazarovici et al., 2006b), neuroprotection (Zacchigna et al., 2008) and cardioprotection (Kreusser et al., 2008; Markel et al., 2008). In view of their dual effects on both neural and vascular systems NGF and VEGF were recently termed angioneurins (Zacchigna et al., 2008). We have recently isolated and characterized both NGF and VEGF-like angioneurin compounds from snake venoms using two steps of reverse phase HPLC followed by protein sequencing. As expected, these growth factors were able to induce *in vivo* plasma extravasation by causing a general microvessel leakage and *in vitro* increased permeability (reduction in electrical resistance) of brain capillary endothelial monolayer (Fig. 4) (Brown et al., 2007). These properties strongly suggest that these angioneurins have an offensive role upon envenomation in improving the pharmacokinetic properties of snake venom neurotoxins and hemorrhagins. In contrast to these destructive properties, snake venom NGF and VEGF potently stimulate endothelial cells proliferation in a TrkA and VEGFR-2 receptors dependent manner, respectively (Lazarovici et al., 2006b). This stimulatory effect of angioneurins on endothelial cells from different vascular beds was correlated with activation of

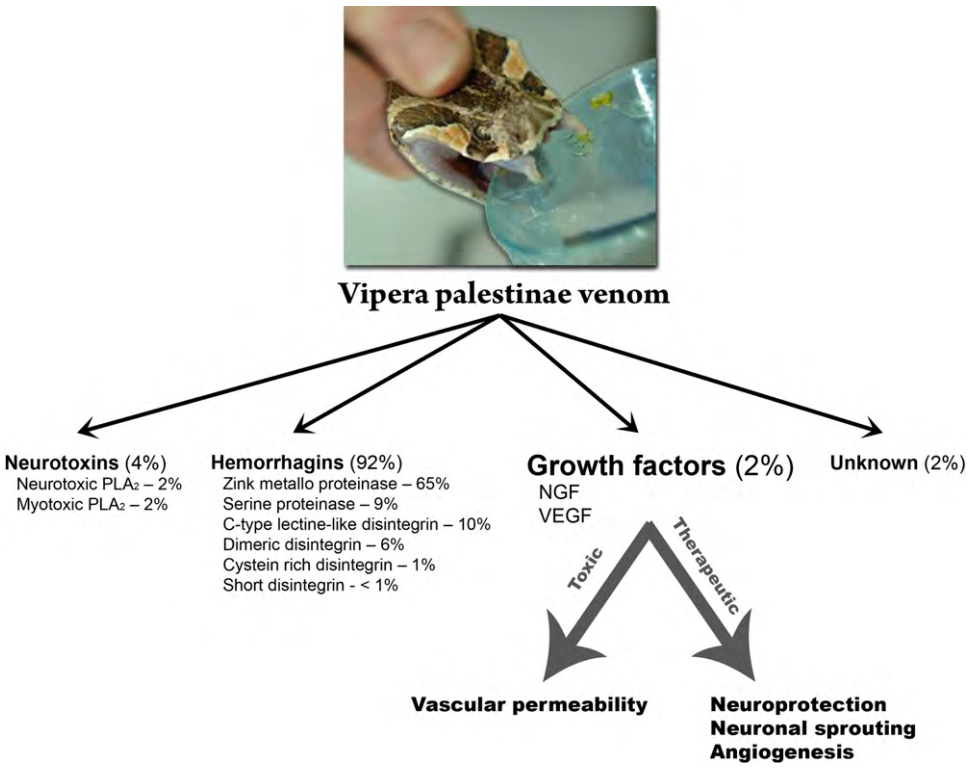

Fig. 4. *Vipera palestinae* venom relative composition of various toxins and growth factors. Growth factors of total venom are further divided by possible toxic and/or therapeutic functions.

MAPK signaling pathway (Cantarella et al., 2002; Lecht et al., 2007). *In vivo*, these angioneurins induced angiogenesis in a quail chorioallantoic membrane assay (Lazarovici et al., 2006a) and in a Matrigel plug assay in mice (Brown et al., 2007). The viper VEGF-like the natural ligand VEGF-A_{165} exert its specific activity by binding to a VEGF receptor type 2 but do not bind to the Flt or neuropilin receptor families (Yamazaki et al., 2003) providing for the first time unique pharmacological tools to study VEGF receptor type 2 function and toward development of novel agonists and antagonists. Similar to snake venom VEGF-induced angiogenic effects, venom-derived NGFs promoted migration (Dolle et al., 2005), capillary sprouting and other angiogenic functions (Lazarovici et al., 2006a). Recently, α9β1 integrin, known to interact with VEGF isoforms, was also found as a receptor for NGF, providing a novel target and explanation for angioneurins-induced migration of endothelial cells (Staniszewska et al., 2008). The unique overexpression of α9β1 integrin in glioblastoma multiforme, which respond to NGF by increased invasiveness, propose anti-NGF therapeutic approach in brain tumors chemotherapy. It is well recognized to date that many tumors overexpress angioneurins and their respective receptors TrkA and VEGF receptor type 2 resulting with increased proliferation and blood supply (angiogenesis) of the tumor (Florenes et al., 2004; Odegaard et al., 2007). Bevacizumab (Avastin®), a neutralizing anti-VEGF antibody FDA-approved for inhibiting angiogenesis and tanezumab a neutralizing anti-NGF antibody in phase II clinical trials for inhibiting metastatic pain and possibly angiogenesis may become important future anticancer drugs.

In conclusion, snake venom angioneurins analogues like the human recombinant ones are important mediators of the physiological cross-talk between the cardiovascular and nervous systems. However, under certain conditions these angioneurins may induce pathologies such as tumor development (chronic effects) or disruption of the vascular barrier function during envenomation (acute effects) (Fig. 4). Furthermore, snake venom angioneurins maybe used as important pharmacological tools and as lead compounds in novel drug discovery, as demonstrated with the first oral angiotensin-converting enzyme inhibitor, captopril, indicated for reducing blood pressure and developed from the Brazilian viper venom (Cohen, 1985) or eptifibatide and tirofiban, derivatives of snake venom disintegrins, which inhibit glycoprotein αIIbβ3 integrin receptors leading to inhibition of platelet aggregation (Marcinkiewicz, 2005).

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From skin irritants to anti-inflammatory peptides

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Introduction

Exposure of mammalian skin to an irritating chemical such as mustard gas (sulfur mustard, SM) results in erythema followed by edema, blister formation and desquamation (Balali-Mood and Hefazi, 2005). The vesicating effect of SM stems from its conversion to the electrophilic ethylene episulfonium in aqueous environment resulting in alkylation of DNA, RNA and proteins, arrest of catabolic and anabolic functions and cell death (Wormser, 1991). It was postulated that nucleophilic agents may possess antidotal effect by chemical neutralization of the reactive mustard electrophile, however, the tested agents were of limited value in protecting skin against mustard toxicity in experimental animals (Casillas et al., 2000).

Effect of iodine on skin burns

We adopted another approach for protecting skin against SM, hypothesizing that SM can be inactivated by oxidation of its sulfur atom to form SM sulfoxide. This derivative was reported to be devoid of vesicating activity (Dixon and Needham, 1946), presumably due to its inability to form the cyclic ethylene episulfonium intermediate. In this view, it was hypothesized that topical treatment with an oxidizing agent may protect skin against SM toxicity. Iodine was an appropriate candidate due to its oxidizing activity and wide use as a safe topical antiseptic. Indeed, post exposure treatment with topical iodine or povidone-iodine (PI, an iodophor which releases iodine in aqueous environment) preparations significantly reduced SM-induced skin lesions in guinea pigs (Fig. 5, Wormser et al., 1997). However, chemical analysis revealed that iodine does not oxidize SM (Wormser et al., 2000), indicating a different mechanism of action for its protective effect. Moreover, iodine had a counter-irritating effect against non-oxidizable dermatotoxic agents such as iodoacetate (Wormser et al., 1997) and hydrofluoric acid (Wormser et al., 2002). Furthermore, topical application of PI or iodine protected against heat burns in both humans (Wormser, 1998) and experimental animals (Wormser et al., 2002). In view of these data, it was postulated that iodine exerts its counter-irritating activity by a pharmacological mechanism that alters skin response to noxious stimuli, resulting in hyposusceptibility of the epidermal and dermal layers to chemical irritants and heat burns.

Mechanism of action of iodine

In order to verify the mechanism of action of iodine, we adopted two different approaches that ultimately led to the same result.

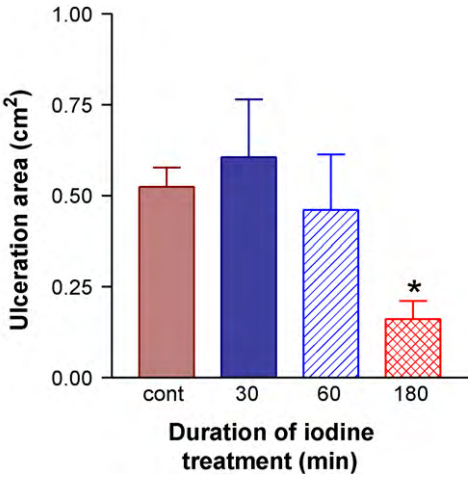

Fig. 5. Effect of duration of iodine application on SM-induced skin toxicity. Shaved backs of guinea pigs were exposed to 1 μl neat SM (6 exposure sites per animal) followed by iodine application (30 min interval). Durations of iodine treatment are indicated. Results are the mean ± SE (cont $n = 30$; $n = 9$ for the rest). $^{*}p < 0.05$.

The first one hypothesized that iodine induces endogenous protective factor(s) in the irritant-exposed skin whereby skin is protected. In order to test this hypothesis guinea pig skin was exposed to heat stimuli followed by topical iodine treatment. Extract of the skin was intradermally injected into naïve guinea pigs. Degree of irritation was significantly reduced as compared to the control animals injected with extract from untreated and unexposed skin. Interestingly, the protective effect of the extract was observed only when irritation was followed by iodine application whereas extract of guinea pigs treated with iodine or burn only had no beneficial activity.

Potential involvement of substance P

The second approach was based on the potential interaction of iodine with inflammatory mediators during the process of skin evolution. Levels of circulatory inflammatory mediators such as IL-1, IL-6 and TNFα were elevated upon thermal injury in rats (Kataranovski et al., 1999). Altered functions of inflammatory cells such as macrophages (Ogle et al., 1997), monocytes (Schwacha et al., 2002) and oxygen radical formation (Demling and Lalonde, 1990; Till et al., 1985) were observed in animals exposed to thermal stimuli. Chemical injuries induced by SM were also reported to involve a variety of inflammatory mediators including proteinases (Woessner et al., 1990), chemotactic factors (Tanaka et al., 1997), cytokines (Tsuruta et al., 1996) and oxidative stress (Elsayed et al., 1992). Inhibition of the inflammatory response reduced the severity of dermal and epidermal lesions caused by SM (Casillas et al., 2000). Among the inflammatory mediators, the undecapeptide substance P (SP) plays an important role in the evolution and progression of skin inflammation (Legat et al., 2002; Walsh et al., 1995). This peptide (SP: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) was shown to be involved in the inflammatory response of chemical (Casbohm et al., 2004) and thermal (Scott et al., 2005) skin burns. Being a potent vasodilator (Paus et al., 2006) and pain mediator (Kingery et al., 2003), SP may serve as an important contributor to the inflammatory process occurring during skin burn.

The dramatic effect of PI on human burns (Wormser, 1998), namely, relatively rapid reduction in pain sensation and prevention of erythema and burn development, led us to the hypothesis that iodine operates by neutralization of SP. The methionine amide residue at the 11th position is a candidate for oxidation to form the corresponding SP sulfoxide which was shown to be less pharmacologically active (Floor and Leeman, 1980) than the parent peptide. Although the oxidizing activity of iodine is too weak to produce SP sulfoxide *in vitro*, our primary assumption was that iodine application might alter tissue reactions, possibly by increasing its oxidative potential, leading to SP oxidation.

The anti-inflammatory peptides III and IIIM1

We realized that SP was oxidized in SM-exposed skin without iodine treatment probably due to the secretion of hydrogen peroxide and other oxidizing agents from the polymorphonuclear cells recruited in the inflamed skin area, as opposed to our primary working hypothesis. Nevertheless as a byproduct of this analysis we discovered a novel peptide fragment of H2A histone 36–44 presumably induced by iodine. The peptide isolated from guinea pig skin (termed peptide III) has the sequence KGNYAERIA while the mouse analog (termed IIIM1) differs in three amino acid residue with the sequence KGHYAERVG. Peptide III reduced skin irritation in the guinea pig skin irritation test whereas IIIM1 ameliorated the inflammatory response in the mouse ear swelling test (Brodsky et al., 2008). Interestingly, cultured HaCaT keratinocytes transfected with the peptide MKGHYAERVG in which methionine residue was added to the N-terminus of the IIIM1 peptide (as an initiation codon) showed 3.1-fold viability to SM as compared to the control cells (Brodsky et al., 2008). The anti-inflammatory effect of the peptide was further demonstrated in the reduction of oxidative burst of activated HL-60 neutrophils. The protective effect of the peptide against oxidative stress was also shown in *in vivo* studies. Skin edema caused by intradermal injection of glucose oxidase (producing hydrogen peroxide from glucose) was significantly reduced by the peptide (Brodsky et al., 2008).

In summary, the anti-inflammatory activity of IIIM1 peptide might be of potential use for skin irritation caused by chemical or heat stimuli and also for dermal disorders such as psoriasis and pemphigus. Moreover, other types of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis might potentially be affected by this nonapeptide. Encouraging preliminary findings were obtained in experimental autoimmune encephalitis (EAE), an animal model of multiple sclerosis. Treatment of diseased mice and rats with the peptide caused reduction in neurological score accompanied with decreased inflammatory and autoimmune responses. In this view it is anticipated that the peptide would be of beneficial effect in prevention and treatment of tissue damage in the central and peripheral nervous systems. This may be applied to SM that was reported to cause neuropsychiatric symptoms and changes in electromyography and nerve conduction velocity (Balali-Mood and Hefazi, 2005). Of particular interest are neurotoxic agents such as organophosphates and metals like manganese and lead whose neuronal damage involves inflammatory response including production and secretion of cytokines (Chapman et al., 2006; Valentino et al., 2007; Park and Park, 2010). Due to its anti-inflammatory and immunomodulatory effects, IIIM1 might be an appropriate candidate for prevention and treatment of adverse effects caused by neurotoxic agents.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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References

American Psychiatric Association. The diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV). Washington, DC: APA; 1994.

Aroch I, Harrus S. Retrospective study of the epidemiological, clinical, haematological and biochemical findings in 109 dogs poisoned by *Vipera xanthina* palestinae. Vet Rec 1999;144:532–5.

Balali-Mood M, Hefazi M. The pharmacology, toxicology, and medical treatment of sulphur mustard poisoning. Fundam Clin Pharmacol 2005;19:297–315.

Banaschewski T, Becker K, Scherag S, Franke B, Coghill D. Molecular genetics of attention-deficit/hyperactivity disorder: an overview. Eur Child Adolesc Psychiatry 2010;19:237–57.

Bar-Ilan I, Shmerkin S, Mingelgrin U, Levanon D. Survey of pesticide distribution in upper Jordan basin. Water Air Soil Pollut 2000;119:139–56.

Bar-Ilan Y, Malman G. In: Migal IC., editor. Usage survey of pesticides in the Lake Kinneret Basin – 2006 period. 2007;1–92.

Barkley RA. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychol Bull 1997;121:65–94.

Bazaa A, Marrakchi N, El Ayeb M, Sanz L, Calvete JJ. Snake venomics: comparative analysis of the venom proteomes of the Tunisian snakes *Cerastes cerastes*, *Cerastes vipera* and *Macrovipera lebetina*. Proteomics 2005;5:4223–35.

Besser R, Guttman L. Intoxication with organophosphorous compounds.In: Vinken PR, Bruyn GW, editors. Handbook of clinical neurology. In: de Wolff FA, editor. Intoxications of the nervous system Part I, vol. 20(64). Amsterdam: Elsevier; 1995. p. 151–79.

Bouchard MF, Bellinger DC, Wright RO, Weisskopf MG. Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. Pediatrics 2010;125:e1270–7.

Bowers MB Jr, Goodman E, Sim VM. Some behavioral changes in man following anticholinesterase administration. J Nerv Ment Dis 1964;138:383–9.

Brodsky B, Erlanger-Rosengarten A, Proscura E, Shapira E, Wormser U. From topical antidote against skin irritants to a novel counter-irritating and anti-inflammatory peptide. Toxicol Appl Pharmacol 2008;229:342–50.

Brown MC, Calvete JJ, Staniszewska I, Walsh EM, Perez-Liz G, Del Valle L, et al. VEGF-related protein isolated from *Vipera palestinae* venom, promotes angiogenesis. Growth Factors 2007;25:108–17.

Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 2000;29:222–30.

Calvete JJ, Sanz L, Angulo Y, Lomonte B, Gutierrez JM. Venoms, venomics, antivenomics. FEBS Lett 2009;583:1736–43.

Cantarella G, Lempereur L, Presta M, Ribatti D, Lombardo G, Lazarovici P, et al. Nerve growth factor-endothelial cell interaction leads to angiogenesis in vitro and in vivo. FASEB J 2002;16:1307–9.

Casbohm SL, Rogers JV, Stonerock MK, Martin JL, Ricketts-Kaminsky KM, Babin MC, et al. Localization of substance P gene expression for evaluating protective countermeasures against sulfur mustard. Toxicology 2004;204:229–39.

Casillas RP, Kiser RC, Truxall JA, Singer AW, Shumaker SM, Niemuth NA, et al. Therapeutic approaches to dermatotoxicity by sulfur mustard. I. Modulation of sulfur mustard-induced cutaneous injury in the mouse ear vesicant model. J Appl Toxicol 2000;20(Suppl. 1):S145–51.

Chapman S, Kadar T, Gilat E. Seizure duration following sarin exposure affects neuroinflammatory markers in the rat brain. Neurotoxicology 2006;27:277–83.

Cohen ML. Synthetic and fermentation-derived angiotensin-converting enzyme inhibitors. Annu Rev Pharmacol Toxicol 1985;25:307–23.

Demling RH, Lalonde C. Systemic lipid peroxidation and inflammation induced by thermal injury persists into the post-resuscitation period. J Trauma 1990;30:69–74.

Dixon M, Needham DM. Biochemical research on chemical warfare agents. Nature 1946;158:432–8.

Dolle JP, Rezvan A, Allen FD, Lazarovici P, Lelkes PI. Nerve growth factor-induced migration of endothelial cells. J Pharmacol Exp Ther 2005;315:1220–7.

Donders J, Tulsky DS, Zhu J. Criterion validity of new WAIS-II subtest scores after traumatic brain injury. J Int Neuropsychol Soc 2001;7:892–8.

Elsayed NM, Omaye ST, Klain GJ, Korte DW Jr. Free radical-mediated lung response to the monofunctional sulfur mustard butyl 2-chloroethyl sulfide after subcutaneous injection. Toxicology 1992;72:153–65.

Estez WK. Learning theory and intelligence. Am Psychol 1974;29:740–9.

Fang YZ, Yang S, Guoyao W. Free radicals, antioxidant, and nutrition. Nutrition 2002;9(18):872–87.

Farahat TM, Abdelrasoul GM, Amr MM, Shebl MM, Farahat FM, Anger WK. Neurobehavioural effects among workers occupationally exposed to organophosphorous pesticides. Occup Environ Med 2003;60:279–86.

Farooqui AA, Yi Ong W, Lu XR, Halliwell B, Horrocks LA. Neurochemical consequences of kainate-induced toxicity in brain: involvement of arachidonic acid release and prevention of toxicity by phospholipase A(2) inhibitors. Brain Res Brain Res Rev 2001;38:61–78.

Finkelstein Y. Obidoxime chloride. In: Dollery CT, editor. Therapeutic drugs, supplement 1. Edinburgh: Churchill Livingstone; 1992; 2144–8.

Finkelstein Y, Kushnir A, Raikhlin-Eisenkraft B, Taitelman U. Antidotal therapy of severe acute organophosphate poisoning: a multihospital study. Neurotoxicol Teratol 1989;11:593–6.

Finkelstein Y, Taitelman U, Biegon A. CNS involvement in acute organophosphate poisoning: specific pattern of toxicity, clinical correlates and antidotal treatment. Ital J Neurol Sci 1988a;9:437–46.

Finkelstein Y, Wolff M, Biegon A. Brain acetylcholinesterase after acute parathion poisoning: a comparative quantitative histochemical analysis post mortem. Ann Neurol 1988b;24:252–7.

Finkelstein Y, Wolff M, Biegon A. Brain acetylcholinesterase after parathion poisoning: a comparative quantitative histochemical analysis post-mortem. Toxicology 1988c;49:165–9.

Floor E, Leeman SE. Substance P sulfoxide: separation from substance P by high-pressure liquid chromatography, biological and immunological activities, and chemical reduction. Anal Biochem 1980;101:498–503.

Florenes VA, Maelandsmo GM, Holm R, Reich R, Lazarovici P, Davidson B. Expression of activated TrkA protein in melanocytic tumors: relationship to cell proliferation and clinical outcome. Am J Clin Pathol 2004;122:412–20.

Fogelman Y, Vinker S, Guy N, Kahan E. Prevalence of and change in the prescription of methylphenidate in Israel over a 2-year period. CNS Drugs 2003;17:915–9.

Gershon S, Shaw FH. Psychiatric sequelae of chronic exposure to organophosphorus insecticides. Lancet 1961;1:1371–4.

Goldfrank L, Flomenbaum N, Lewin N, Weisman R, Howland MA, Kaul B. Anticholinergic poisoning. J Toxicol Clin Toxicol 1982;19:17–25.

Gregory RI. In: Gregory RJ, editor. Psychological testing: history, principles, and applications 5th ed. Boston: Pearson; 2007 p. 203–4.

Grob D, Harvey AM. The effects and treatment of nerve gas poisoning. Am J Med 1953;14:52–63.

Gunnell D, Eddleston M. Suicide by intentional ingestion of pesticides: a continuing tragedy in developing countries. Int J Epidemiol 2003;32:902–9.

Heaton KJ, Palumbo CL, Proctor SP, Killiany RJ, Yurgelun-Todd DA, White RF. Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. Neurotoxicology 2007;28:761–9.

Heinemann U, Buchheim K, Gabriel S, Kann O, Kovacs R, Schuchmann S. Cell death and metabolic activity during epileptiform discharges and status epilepticus in the hippocampus. Prog Brain Res 2002;135:197–210.

Ilani S, Goldsmith JR, Israeli R. Neurotoxicity of organophosphate insecticides among a Negev population with long-term exposures. In: Hogstedt E, Reuterwall C, editors. Progress in occupational epidemiology (Sixth Latl symposium on epidemiology in occupational health), vol. 4. Stockholm: Elsevier; 1988. p. 253–62.

Israel National Poison Control Center. 1987. Final report on treatment of 53 severe acute OP poisoning in Israel. A part of international epidemiological study on low-level, long-term health effects of organophosphorous pesticides, supported by the United Nations Development Programme and the WHO Regional Office for Europe 1987.

Kamel F, Rowland AS, Park LP, Anger WK, Baird DD, Gladen BC, et al. Neurobehavioral performance and work experience in Florida farmworkers. Environ Health Perspect 2003;111:1765–72.

Kataranovski M, Magic Z, Pejnovic N. Early inflammatory cytokine and acute phase protein response under the stress of thermal injury in rats. Physiol Res 1999;48:473–82.

Kaufmann R, Goldberg-Stern H, Shuper A. Attention-deficit disorders and epilepsy in childhood: incidence, causative relations and treatment possibilities. J Child Neurol 2009;24:727–33.

Kingery WS, Davies MF, Clark JD. A substance P receptor (NK1) antagonist can reverse vascular and nociceptive abnormalities in a rat model of complex regional pain syndrome type II. Pain 2003;104:75–84.

Kisiel DG, Calvete JJ, Katzhendler J, Fertala A, Lazarovici P, Marcinkiewicz C. Structural determinants of the selectivity of KTS-disintegrins for the alpha1beta1 integrin. FEBS Lett 2004;577:478–82.

Kochunov P, Coyle T, Lancaster J, Robin DA, Hardies J, Kochunov V, et al. Processing speed is correlated with cerebral health markers in the frontal lobes as quantified by neuroimaging. Neuroimage 2010;49:1190–9.

Koeller WC, Klawans HL. Organophosphorus introxication. In: Vinken PJ, Bruyn GW, editors. Handbook of clinical neurology, vol. 37. Amsterdam: Elsevier/North Holland Biomedical Press; 1979. p. 541–62.

Kofman O, Berger A, Massarwa A, Friedman A, Jaffar AA. Motor inhibition and learning impairments in school-aged children following exposure to organophosphate pesticides in infancy. Pediatr Res 2006;60:88–92.

Korkman M, Kemp SL, Kirk U. Effects of age on neurocognitive measures of children ages 5 to 12: a cross-sectional study on 800 children from the United States. Dev Neuropsychol 2001;20:331–54.

Kostiza T, Dahinden CA, Rihs S, Otten U, Meier J. Nerve growth factor from the venom of the Chinese cobra Naja naja atra: purification and description of non-neuronal activities. Toxicon 1995;33:1249–61.

Kreusser MM, Buss SJ, Krebs J, Kinscherf R, Metz J, Katus HA, et al. Differential expression of cardiac neurotrophic factors and sympathetic nerve ending abnormalities within the failing heart. J Mol Cell Cardiol 2008;44:380–7.

Krizaj I, Bdolah A, Gubensek F, Bencina P, Pungercar J. Protein and cDNA structures of an acidic phospholipase A2, the enzymatic part of an unusual, two-component toxin from *Vipera palaestinae*. Biochem Biophys Res Commun 1996;227:374–9.

Kwong TC. Organophosphate pesticides: biochemistry and clinical toxicology. Ther Drug Monit 2002;24:144–9.

Lafon-Cazal M, Pietri S, Culcasi M, Bockaert J. NMDA-dependent superoxide production and neurotoxicity. Nature 1993;364:535–7.

Lallement G, Carpentier P, Pernot-Marino I, Baubichon D, Collet A, Blanchet G. Involvement of the different rat hippocampal glutamatergic receptors in development of seizures induced by soman: an autoradiographic study. Neurotoxicology 1991;12:655–64.

Lazarovici P, Gazit A, Staniszewska I, Marcinkiewicz C, Lelkes PI. Nerve growth factor (NGF) promotes angiogenesis in the quail chorioallantoic membrane. Endothelium 2006a;13:51–9.

Lazarovici P, Marcinkiewicz C, Lelkes PI. Cross talk between the cardiovascular and nervous systems: neurotrophic effects of vascular endothelial growth factor (VEGF) and angiogenic effects of nerve growth factor (NGF) – implications in drug development. Curr Pharm Des 2006b;12:2609–22.

Lecht S, Cohen G, Marcinkiewicz C, Lazarovici P. Identification and pharmacological characterization of novel nerve growth factor isoform from *Echis carinatus sochureki* snake venom. Israel: Ma'ale Hachamisha; 2008.

Lecht S, Puxeddu I, Levi-Schaffer F, Reich R, Davidson B, Schaefer E, et al. Nerve growth factor – a neurotrophin with angiogenic activity. In: Maragudakis M, editor. Angiogenesis: basic science and clinical applications. Kerala: Transworld Research Network; 200799–113.

Legat FJ, Griesbacher T, Schicho R, Althuber P, Schuligoi R, Kerl H, et al. Repeated subinflammatory ultraviolet B irradiation increases substance P and calcitonin gene-related peptide content and augments mustard oil-induced neurogenic inflammation in the skin of rats. Neurosci Lett 2002;329:309–13.

Leibson T, Lifshitz M. Organophosphate and carbamate poisoning: review of the current literature and summary of clinical and laboratory experience in southern Israel. Isr Med Assoc J 2008;10:767–70.

Lifshitz M, Rotenberg M, Sofer S, Tamiri T, Shahak E, Almog S. Carbamate poisoning and oxime treatment in children: a clinical and laboratory study. Pediatrics 1994;93:652–5.

Lifshitz M, Shahak E, Sofer S. Carbamate and organophosphate poisoning in young children. Pediatr Emerg Care 1999;15:102–3.

Lipton SA. The molecular basis of memantine action in Alzheimer's disease and other neurologic disorders: low-affinity, uncompetitive antagonism. Curr Alzheimer Res 2005;2:155–65.

Lotti M. Clinical toxicology of anticholinesterase agents in humans. In: Krieger RI, editor. Handbook of pesticide toxicology. San Diego: Academic Press; 20011043–85.

Luria AR. The functional organization of the brain. Sci Am 1970;222:66–72.

Luria AR, Karasseva TA. Disturbances of auditory speech memory in focal lesions of the deep regions of the left temporal lobe. Neuropsychologia 1968;6:97–104.

Marcinkiewicz C. Functional characteristic of snake venom disintegrins: potential therapeutic implication. Curr Pharm Des 2005;11:815–27.

Markel TA, Wang Y, Herrmann JL, Crisostomo PR, Wang M, Novotny NM, et al. VEGF is critical for stem cell-mediated cardioprotection and a crucial paracrine factor for defining the age threshold in adult and neonatal stem cell function. Am J Physiol Heart Circ Physiol 2008;295:H2308–14.

McDonald BE, Costa LG, Murphy SD. Spatial memory impairment and central muscarinic receptor loss following prolonged treatment with organophosphates. Toxicol Lett 1988;40:47–56.

Meng Z, Grauer F, Ben-Michael E, Richter ED. Alkyl phosphates and cholinesterase in workers exposed to organophosphate pesticides. In: Proceedings of the 6th international conference of the Israeli Society for ecological and environmental quality sciences VIA. 1996;283–7.

Mesulam MM. Attention, confusional states and neglect. In: Mesulam MM, editor. Principles of behavioral neurology. Philadelphia: Davis; 1985125–68.

Mesulam MM, Volicer L, Marquis JK, Mufson EJ, Green LC. Systematic regional differences in the cholinergic innervation of the primate cerebral cortex: distribution of enzyme activities and some behavioral implications. Ann Neurol 1986;19:144–51.

Metcalf DR, Holmes JH VII. Toxicology and physiology. EEG, psychological, and neurological alterations in humans with organophosphorus exposure. Ann N Y Acad Sci 1969;160:357–65.

Milatovic D, Aschner M. Measurement of Isoprostanes as Markers of Oxidative Stress in Neuronal Tissue. Curr Protoc Toxicol 2009;12:1–12 unit 12.14.

Milatovic D, Zaja-Milatovic S, Montine KS, Horner PJ, Montine TJ. Pharmacologic suppression of neuronal oxidative damage and dendritic degeneration following direct activation of glial innate immunity in mouse cerebrum. J Neurochem 2003;87:1518–26.

Milatovic S, Gupta RC, Aschner M, Montine TJ, Milatovic D. Pharmacologic suppression of oxidative damage and dendritic degeneration following kainic acid-induced excitotoxicity in mouse cerebrum. Neurotoxicology 2008;29:621–7.

Moroz C. *Vipera palaestinae* antivenin. Public Health Rev 1998;26:233–6.

Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci USA 1990;87:9383–7.

Namba T, Nolte CT, Jackrel J, Grob D. Poisoning due to organophosphate insecticides. Acute and chronic manifestations. Am J Med 1971;50:475–92.

Nicholls DG, Vesce S, Kirk L, Chalmers S. Interactions between mitochondrial bioenergetics and cytoplasmic calcium in cultured cerebellar granule cells. Cell Calcium 2003;34:407–24.

Odegaard E, Staff AC, Abeler VM, Kopolovic J, Onsrud M, Lazarovici P, et al. The activated nerve growth factor receptor p-TrkA is selectively expressed in advanced-stage ovarian carcinoma. Hum Pathol 2007;38:140–6.

Ogle CK, Valente JF, Guo X, Li BG, Ogle JD, Alexander JW. Thermal injury induces the development of inflammatory macrophages from nonadherent bone marrow cells. Inflammation 1997;21:569–82.

Ozsuer H, Gorgulu A, Kiris T, Cobanoglu S. The effects of memantine on lipid peroxidation following closed-head trauma in rats. Neurosurg Rev 2005;28:143–7.

Park EJ, Park K. Induction of oxidative stress and inflammatory cytokines by manganese chloride in cultured T98G cells, human brain glioblastoma cell line. Toxicol In Vitro 2010;24:472–9.

Parron T, Hernandez AF, Pla A, Villanueva E. Clinical and biochemical changes in greenhouse sprayers chronically exposed to pesticides. Hum Exp Toxicol 1996;15:957–63.

Pastor PN, Reuben CA. Diagnosed attention deficit hyperactivity disorder and learning disability: United States, 2004–2006. National Center for Health Statistics. Vital Health Stat 2008;10:237.

Patel MN. Oxidative stress, mitochondrial dysfunction, and epilepsy. Free Radic Res 2002;36:1139–46.

Paus R, Theoharides TC, Arck PC. Neuroimmunoendocrine circuitry of the 'brain–skin connection'. Trends Immunol 2006;27:32–9.

Prendergast MA, Terry AV Jr, Buccafusco JJ. Effects of chronic, low-level organophosphate exposure on delayed recall, discrimination, and spatial learning in monkeys and rats. Neurotoxicol Teratol 1998;20:115–22.

Proctor SP, Heaton KJ, Heeren T, White RF. Effects of sarin and cyclosarin exposure during the 1991 Gulf War on neurobehavioral functioning in US army veterans. Neurotoxicology 2006;27:931–9.

Prody CA, Dreyfus P, Zamir R, Zakut H, Soreq H. De novo amplification within a "silent" human cholinesterase gene in a family subjected to prolonged exposure to organophosphorous insecticides. Proc Natl Acad Sci U S A 1989;86:690–4.

Reidy TJ, Bowler RM, Rauch SS, Pedroza GI. Pesticide exposure and neuropsychological impairment in migrant farm workers. Arch Clin Neuropsychol 1992;7:85–95.

Reitan RM. The relation of the trail making test to organic brain damage. J Consult Psychol 1955;19:393–4.

Richter ED. Evaluation of the pilot phase of the epidemiological study on the health effects of organophosphorous pesticides. Organophosphorous pesticides an epidemiological study EH 22, vol. 138. Copenhagen: WHO-Europe; 1987 p. 1–32.

Richter ED. Organophosphorus pesticides – a multinational epidemiological study. Copenhagen: WHO-Europe; 1993 (monograph).

Richter ED, Chuwers P, Levy Y, Gordon M, Grauer F, Marzouk J, et al. Health effects from exposure to organophosphate pesticides in workers and residents in Israel. Isr J Med Sci 1992a;28:584–98.

Richter ED, Kaspi L, Gordon M, Levy S, Israeli R, Gruener N. Monitoring for neurotoxic effects from low level exposures to organophosphate pesticides. Sci Total Environ 1984;32:335–44.

Richter ED, Kowalski M, Leventhal A, Grauer F, Marzouk J, Brenner S, et al. Illness and excretion of organophosphate metabolites four months after household pest extermination. Arch Environ Health 1992b;47:135–8.

Richter ED, Rosenvald Z, Kaspi L, Levy S, Gruener N. Sequential cholinesterase tests and symptoms for monitoring organophosphate absorption in field workers and in persons exposed to pesticide spray drift. Toxicol Lett 1986;33:25–35.

Roldán-Tapia L, Parrón T, Sánchez-Santed F. Neuropsychological effects of long-term exposure to organophosphate pesticides. Neurotoxicol Teratol 2005;27:259–66.

Rosenstock L, Keifer M, Daniell WE, McConnell R, Claypoole K. The Pesticide Health Effects Study GroupChronic central nervous system effects of acute organophosphate pesticide intoxication. Lancet 1991;338:223–7.

Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J, McCauley L. Organophosphate pesticide exposure and neurobehavioral performance in agricultural and non-agricultural Hispanic workers. Environ Health Perspect 2006;114:691–6.

Rowntree DW, Nevin S, Wilson A. The effects of diisopropylfluorophosphonate in schizophrenia and manic depressive psychosis. J Neurol Neurosurg Psychiatry 1950;13:47–62.

Ruckart PZ, Kakolewski K, Bove FJ, Kaye WE. Long-term neurobehavioral health effects of methyl parathion exposure in children in Mississippi and Ohio. Environ Health Perspect 2004;112:46–51.

Savage EP, Keefe TJ, Mounce LM, Heaton RK, Lewis JA, Burcar PJ. Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch Environ Health 1988;43:38–45.

Schwacha MG, Schneider CP, Chaudry IH. Differential expression and tissue compartmentalization of the inflammatory response following thermal injury. Cytokine 2002;17:266–74.

Scott JR, Muangman PR, Tamura RN, Zhu KQ, Liang Z, Anthony J, et al. Substance P levels and neutral endopeptidase activity in acute burn wounds and hypertrophic scar. Plast Reconstr Surg 2005;115:1095–102.

Sidell FR, Hurst CG. Long-term health effects of nerve agents and mustard. In: Zajtchuk R, Bellamy RF, editors. Textbook of military medicine – medical aspects of chemical and biological warfare. Washington, DC: Office of the Surgeon General, Department of Army; 1977229–46.

Sofer S, Tal A, Shahak E. Carbamate and organophosphate poisoning in early childhood. Pediatr Emerg Care 1989;5:222–5.

Staniszewska I, Sariyer IK, Lecht S, Brown MC, Walsh EM, Tuszynski GP, et al. Integrin alpha9 beta1 is a receptor for nerve growth factor and other neurotrophins. J Cell Sci 2008;121:504–13.

Steenland K. Chronic neurological effects of organophosphate pesticides. BMJ 1996;312:1312–3.

Steenland K, Jenkins B, Ames RG, O'Malley M, Chrislip D, Russo J. Chronic neurological sequelae to organophosphate pesticide poisoning. Am J Public Health 1994;84:731–6.

Stephens R, Spurgeon A, Calvert IA, Beach J, Levy LS, Berry H, et al. Neuropsychological effects of long-term exposure to organophosphates in sheep dip. Lancet 1995;345:1135–9.

Stoller A, Krupinski J, Christophers AJ, Blanks GK. Organophosphorus insecticides and major mental illness: an epidemiological investigation. Lancet 1965;i:1387–8.

Tanaka F, Dannenberg AM Jr, Higuchi K, Nakamura M, Pula PJ, Hugli TE, et al. Chemotactic factors released in culture by intact developing and healing skin lesions produced in rabbits by the irritant sulfur mustard. Inflammation 1997;21:251–67.

Thwin MM, Gopalakrishnakone P. Snake envenomation and protective natural endogenous proteins: a mini review of the recent developments (1991–1997). Toxicon 1998;36:1471–82.

Till GO, Hatherill JR, Tourtellotte WW, Lutz MJ, Ward PA. Lipid peroxidation and acute lung injury after thermal trauma to skin. Evidence of a role for hydroxyl radical. Am J Pathol 1985;119:376–84.

Tsuruta J, Sugisaki K, Dannenberg AM Jr, Yoshimura T, Abe Y, Mounts P. The cytokines NAP-1 (IL-8), MCP-1, IL-1 beta, and GRO in rabbit inflammatory skin lesions produced by the chemical irritant sulfur mustard. Inflammation 1996;20:293–318.

Uylings HB, de Brabander JM. Neuronal changes in normal human aging and Alzheimer's disease. Brain Cogn 2002;49:268–76.

van Acker SA, Koymans LM, Bast A. Molecular pharmacology of vitamin E: structural aspects of antioxidant activity. Free Radic Biol Med 1993;15:311–28.

Valentino M, Rapisarda V, Santarelli L, Bracci M, Scorcelletti M, Di Lorenzo L, et al. Effect of lead on the levels of some immunoregulatory cytokines in occupationally exposed workers. Hum Exp Toxicol 2007;26:551–6.

Wade JV, Samson FE, Nelson SR, Pazdernik TL. Changes in extracellular amino acids during soman- and kainic acid-induced seizures. J Neurochem 1987;49:645–50.

Walsh DT, Weg VB, Williams TJ, Nourshargh S. Substance P-induced inflammatory responses in guinea-pig skin: the effect of specific NK1 receptor antagonists and the role of endogenous mediators. Br J Pharmacol 1995;114:1343–50.

Weinbroum AA. Pathophysiological and clinical aspects of combat anticholinesterase poisoning. Br Med Bull 2004;72:119–33.

Weinstein S, Dart R, Staples A, White J. Envenomations: an overview of clinical toxinology for the primary care physician. Am Fam Physician 2009;80:793–802.

Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. Nature 2005;437:497–504.

Weissmann-Brenner A, Friedman LM, David A, Vidan A, Hourvitz A. Organophosphate poisoning: a multihospital survey. Isr Med Assoc J 2002;4:573–6.

Wesseling C, Keifer M, Ahlbom A, McConnell R, Moon JD, Rosenstock L, et al. Long-term neurobehavioral effects of mild poisonings with organophosphate and n-methyl carbamate pesticides among banana workers. Int J Occup Environ Health 2002;8:27–34.

Woessner JF Jr, Dannenberg AM Jr, Pula PJ, Selzer MG, Ruppert CL, Higuchi K, et al. Extracellular collagenase, proteoglycanase and products of their activity, released in organ culture by intact dermal inflammatory lesions produced by sulfur mustard. J Invest Dermatol 1990;95:717–26.

World Resources Institute. People and the environment: resource consumption, population growth, women. New York: Oxford University Press; 1994–1995 p. 116–7.
Wormser U. Toxicology of mustard gas. Trends Pharmacol Sci 1991;12:164–7.
Wormser U. Early topical treatment with povidone-iodine ointment reduces, and sometimes prevents, skin damage following heat stimulus. Burns 1998; 24:383.
Wormser U, Brodsky B, Green BS, Arad-Yellin R, Nyska A. Protective effect of povidone-iodine ointment against skin lesions induced by sulphur and nitrogen mustards and by non-mustard vesicants. Arch Toxicol 1997;71:165–70.
Wormser U, Sintov A, Brodsky B, Amitai Y, Nyska A. Protective effect of topical iodine preparations upon heat-induced and hydrofluoric acid-induced skin lesions. Toxicol Pathol 2002;30:552–8.
Wormser U, Sintov A, Brodsky B, Nyska A. Topical iodine preparation as therapy against sulfur mustard-induced skin lesions. Toxicol Appl Pharmacol 2000;169:33–9.
Yamazaki Y, Takani K, Atoda H, Morita T. Snake venom vascular endothelial growth factors (VEGFs) exhibit potent activity through their specific recognition of KDR (VEGF receptor 2). J Biol Chem 2003;278:51985–8.
Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, et al. Chronic neurobehavioral and central and autonomic nervous system effects of Tokyo subway sarin poisoning. J Physiol Paris 1998;92:317–23.
Zacchigna S, Lambrechts D, Carmeliet P. Neurovascular signalling defects in neurodegeneration. Nat Rev Neurosci 2008;9:169–81.
Zaja-Milatovic S, Gupta RC, Aschner M, Milatovic D. Protection of DFP-induced oxidative damage and neurodegeneration by antioxidants and NMDA receptor antagonist. Toxicol Appl Pharmacol 2009;240:124–31.