Food and Chemical Toxicology 49 (2011) 1152–1159

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Concomitant exposure to arsenic and organophosphates on tissue oxidative stress in rats

Nidhi Dwivedi, Swaran J.S. Flora *

Division of Pharmacology and Toxicology, Defence Research and Development Establishment, Jhansi Road, Gwalior 474 002, India

ARTICLE INFO

Article history:
Received 25 November 2010
Accepted 14 February 2011
Available online 22 February 2011

Keywords:
Arsenic
Dichlorovos
Monocrotophos
Interaction
Oxidative stress
Haematological variables

ABSTRACT

Increased use of organophosphates (OPs) and ever increasing arsenic levels in drinking water and their co-existence in the environment could be potentially hazardous. The present study examines the effects of dichlorvos (DDVP) or monocrotophos (MCP) and sodium meta arsenite, individually or in combination for 16 weeks on variables indicative of hematological and tissue oxidative injury in rats. Co-exposure to DDVP, MCP or arsenic produced significant inhibition of brain and serum AChE levels suggesting synergism. Significant increase in hepatic reactive oxygen species and brain thiobarbituric acid reactive substances was observed in arsenic and OPs exposed animals. Co-exposure to arsenic and OPs exhibited synergism in case of ROS while antagonism was noted in case of TBARS. Serum transaminases increased significantly on exposure to OPs and arsenic suggesting liver injury which was less pronounced in case of co-exposure to DDVP and arsenic. WBC counts too showed less pronounced increase on co-exposure to arsenic with OPs compared to all other exposure. Blood arsenic level decreased on co-exposure to arsenic with OPs. The present study points to some interesting observations regarding interaction between arsenic and organophosphates. While, exposure to arsenic, DDVP and MCP lead to significant oxidative stress, their co-exposure not necessarily produce synergistic effects.

1. Introduction

The contamination of arsenic in natural water is a global problem and has become a challenge for environmental health researchers. The worst affected countries in the world in recent years include India and Bangladesh (Chakraborti et al., 2002). Beside the natural sources of arsenic contamination in drinking water, use of arsenic-containing herbicides, insecticides, pesticides, rodenticides, and preservatives are enough to challenge the aquatic environment as well as humankind (Florea and Busselberg, 2006). One of the reported mechanisms of arsenic toxicity is the inhibition of sulfhydryl-group-containing cellular enzymes (Mishra et al., 2008). Sodium arsenite treatment has been shown to significantly decrease glutathione (GSH) and superoxide dismutase (SOD) activity, decrease glutathione peroxidase activity, and significantly decrease catalase activity. Arsenic exerts its toxicity by generating reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals during its metabolism which results in the oxidative damage and depresses the antioxidant defense system (Liu et al., 2000).

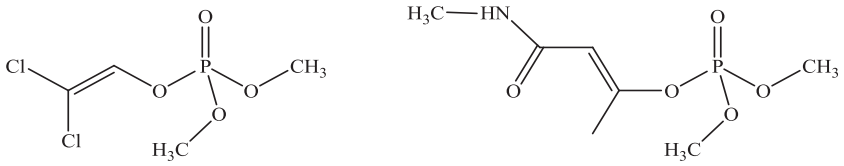
For centuries, several hundred pesticides have been used in agriculture to enhance food productivity by exterminating harmful pests and these pesticides release into the environment and come in contact with human directly or indirectly. The indiscriminate use of these pesticides has caused severe hazardous effects to the humans and animals which results in acute and chronic health problems. Among the wide variety of other environmental pollutants, organophosphorous compounds are extensively used in agriculture, medicine, industry, accidental and suicidal poisoning. OP's are potent neurotoxic chemicals and their substantive application leads to the common occurrence of OP residues in food crops, natural water system, soil and atmosphere, which will be the major sources of exposure (Foreman et al., 2000; Schipper et al., 2008) (see Fig. 1)

Dichlorvos [2,2-dichlorovinyl dimethyl phosphate; DDVP] belonging to organophosphorous pesticides, is a relatively non-persistent organophosphate (OP) compound that undergoes fast and complete hydrolysis in most environmental compartments and is rapidly degraded by mammalian metabolism (WHO, 1989). DDVP shows its effect by directly inhibiting AChE enzyme and accumulating acetylcholine (ACh) which results in the disruption to the nervous and muscular system. (US EPA, 2000).

Abbreviations: DDVP, Dichlorvos; MCP, Monocrotophos; AST, Serum aspartate amino transferase; ALT, Alanine amino transferase (ALT); ROS, Reactive oxygen species; AChE, Acetyl cholinesterase (AChE); GSH, Reduced glutathione; GSSG, Oxidized glutathione; As, Arsenic.

* Corresponding author. Tel.: +91 751 2344301; fax: +91 751 2341148.
E-mail address: sjsflora@drde.drdo.in (S.J.S. Flora).

0278-6915/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2011.02.007



Dichlorovos, DDVP Monocrotophos, MCP

Fig. 1. The organophosphorus.

Another widely used OP compound is Monocrotophos [dimethyl (E)-1-methyl-2-(methyl carbamoyl) vinyl phosphate; MCP], commonly known as Azodrin, widely used throughout the world including India and classified as extremely hazardous pesticide (Yaduvanshi et al., 2010). MCP like other OP has also been found to be a potent neurotoxicant in different organisms and inhibits AChE showing similar symptoms as other OP compounds. MCP and DDVP are widely used OP pesticides and the largest selling agrochemicals in India (Banu et al., 2001). Both DDVP and MCP possess different characteristics and these compounds produce varying degree of toxic effects. Pesticides are lipophilic in nature as they interact with living organisms through the lipid-rich biomembranes and can disturb the balance between prooxidants and antioxidants in the body and damage membrane as a result of lipid peroxidation (Abdollahi et al., 2004). Organophosphorus pesticides and arsenic are known to produce reactive oxygen species (ROS) and there is sufficient information available for the individual effects of toxic chemicals with multiple chemical exposures are believed to represent an actual picture of the human and animal chemical toxic burden. Co-exposure to arsenic and Malathion was hazardous to physical variables based on body or liver weights whilst producing biochemical changes comparable to those caused by the individual agents who pointed at no specific toxicological interaction between the metal and pesticide (Aggarwal et al., 2008). Interaction between pesticides and heavy metals have been reported following changes observed in basic toxicological, haematological and certain immune function parameters (Institoris et al., 1999, 2001). Sub-acute exposure with carbamate pesticide propoxur and cadmium in male Wistar rats altered the neurotoxicological (spontaneous and stimulus-evoked cortical activity, nerve conduction velocity) parameters (Institoris et al., 2002). In another investigation combined administration of dimethoate and arsenic resulted in changes of relative liver weights and mean cell volume (MCV) values.

In case of co exposure, one toxicant may modify the effect of other by altering its kinetics or dynamics. In various parts of the world, arsenic is found in the water at concentration much higher than the WHO permissible level (IPCS, 2001; WHO, 2004). On the other hand continuous use of pesticides is polluting the environment as they never reach their target organisms but are released in air, water and soil (Repetto and Baliga, 1996). Considering the fact that different pesticides and heavy metal are simultaneously being used, there is a possibility of population getting exposed to these pollutants through drinking water. Although numerous reports are available on the individual effects of arsenic and these organophosphates, their combined exposure has not been studied much which may cause more severe toxic effects.

The present study thus was planned to assess the toxic effects of DDVP, MCP and arsenic individually and concomitantly on biochemical variables suggestive of alterations in blood, brain and hepatic oxidative stress. Arsenic concentration in blood and soft tissues too was determined. Another aspect of the study was to evaluate changes in AChE activity due to co-exposure of arsenic and the organophosphorus pesticide.

2. Material and methods

2.1. Chemicals and reagents

The two organophosphorous compounds DDVP (Nuvan 76%), MCP (Kadett-36) were obtained from Syngenta chemicals and P.I. Industries Ltd. (India) respectively and Sodium m- arsenite ($NaAsO_2$, molecular weight 129.9) were procured from Sigma Chemical (USA). All other analytical laboratory chemicals and reagents were purchased from Merck (Germany), Sigma (USA) or BDH chemicals (Mumbai, India). Ultra pure water prepared by Millipore (New Delhi, India) was used through out the experiment to avoid metal contamination and for the preparation of reagents and buffers used for various biochemical assays in our study.

2.2. Animals and treatments

Male Wistar rats (110–120 g) were obtained from Defence Research and Development Establishment (DRDE) animal facility and prior to use, were acclimatized for 7 days 12 h light/dark cycle. The Animal Ethical Committee of DRDE, Gwalior, India, approved the protocols for the experiments. The animals were housed in stainless steel cages in an air-conditioned room with temperature maintained at 25 ± 2 °c. Rats were allowed standard chow diet (Ashirwad Feeds, Chandigarh, India; metal content of diet, in ppm dry weight: Cu 10.0, Zn 45.0, Mn 55.0, Co 5.0, Fe 75.0) throughout the experiment and water ad libitum. Forty animals were randomized into eight groups of five rats each and were treated as below for 16 weeks:

- Group I: Normal (received normal water)
- Group II: DDVP (2.5 mg/kg, subcutaneously)
- Group III: MCP (2.3 mg/kg, orally)
- Group IV: Arsenic as sodium meta arsenite (50 ppm in drinking water)
- Group V: DDVP + MCP (2.5 mg/kg, s.c. + 2.3 mg/kg, orally)
- Group VI: DDVP + Arsenic (as in group II and IV, respectively)
- Group VII: MCP + Arsenic (as in group III and IV, respectively)
- Group VIII: DDVP + MCP + As (as in group II, III and IV, respectively.)

The doses were selected based on our earlier publications (Dwivedi et al., 2010; Flora et al., 2009a). After 16 weeks, blood was collected in heparinized and for serum in non-heparinized tubes separated for various biochemical and hematological parameters. Animals were then euthanized by decapitation. Brain and liver were removed, rinsed in cold saline, blotted, weighed and used for various biochemical variables and metal analysis. Half portion of the liver and brain from each rat was processed immediately for biochemical estimation and the remaining was stored at −20 °C before wet acid digestion with HNO_3 for estimation of arsenic contents.

2.3. Biochemical assays

2.3.1. Clinical hematological variables

Level of mean cell volume (MCV), hematocrit (HCT), hemoglobin (HGB), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets (PLT), red blood cell (RBCs) count and white blood cell (WBCs) count were measured using a Sysmex hematology analyzer (model K4500).

2.3.2. Reactive oxygen species (ROS) level in blood

Amount of ROS in blood was measured using 2′, 7′-dichlrofluorescin diacetate (DCF-DA) that gets converted into highly fluorescent DCF by cellular peroxides (including hydrogen peroxide). The assay was performed as described by Socci et al., 1999. Briefly, 5% RBCs hemolysate was prepared and diluted to 1.5% with ice-cold 40 mM Tris–HCl (pH 7.4). The samples were divided into two equal fractions. In one fraction 40 μL of 1.25 mM DCF-DA in methanol was added for ROS estimation. Another fraction in which 40 μL of methanol was added, served as a control for hemolysate auto fluorescence. All samples were incubated for 15 min in a 37 °C water bath. Fluorescence was determined at 488 nm excitation and 525 nm emission using a fluorescence plate reader (Perkin Elmer, LS-55, UK).