Int J Clin Exp Med 2015;8(10):17891-17901
www.ijcem.com /ISSN:1940-5901/IJCEM0012894

Original Article

Protective effects of the antihistamine promethazine aginst acute paraxon-methyl and dicrotophos toxicity in adult rats

Syed M Nurulain, Shreesh Ojha, Mohammad Shafiullah, Nadia Khan, Murat Oz, Bassem Sadek

Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, P.O. Box 17666, UAE

Received July 14, 2015; Accepted October 9, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: Organophosphorus compound poisoning (OPC) is a global issue. The problem is aggravated with the threats of terrorist use, unintentional use and irresponsible practice as happened recently in turmoil countries. The purpose of the current study was to investigate the old-generation antihistamine promethazine (PROM), a drug with multi pharmacological actions, as an antidote to extremely and highly toxic (WHO's class IA and IB) OPC poisoning in experimental animal models conducted on adult male wistar rats. Experimental groups were treated intraperitoneal (i.p.) with LD_{70} of methyl paraoxon (MPOX), class IA and dicrotophos (DCP), class IB alone and a combination of simultaneously i.p. injection of PROM. Mortality was recorded at 30 minutes, 1, 2, 3, 4, 24, 48 hours post injections. RBC-AChE was measured in survivals. MPOX was chosen for further studies with atropine (ATR) and pralidoxime (PAM). In addition to Kaplan-Meir survival analysis, serum lactate dehydrogenase (LDH) and creatinine kinase (CK) from serum were measured in all experimental groups with MPOX. The results revealed significant protection by PROM in both MPOX and DCP intoxicated rats, though the inhibition of RBC-AChE was high. The observed results show that groups treated with a combination of MPOX and PROM or MPOX, PROM, and PAM were protected higher than those treated with MPOX and ATR or MPOX, ATR, and PAM though statistically not significantly different ($P \leq 0.05$). No effect was observed on the activity of LDH and CK. The study concludes that PROM may be effectively used in OPC poisoning. However, risk/benefits trials and further studies with different doses and other OPC groups are warranted.

Keywords: Promethazine, antihistamine, paraoxon, dicrotophos, atropine, pralidoxime, organophosphorus poisoning

Introduction

Organophosphorus compound (OPC) poisoning is a global issue. The compounds have a wide variety of applications. Thousands of casualties and death are reported each year due to unintentional and intentional use, apart from undesirable exposure in the environment, particularly in agriculture sector. Situation further intensifies by the terrorist use of these compounds which happened several times in the past [1-3]. The use of sarin was suspected in the Syria turmoil recently (August 2013) where thousands of casualties were reported (http://www.theguardian.com/world/2014/mar/06/sarin-gas-attack-civilians-syria-government-un. Last accessed 14.7.15). OPC nerve agents pose a permanent threat for the civilian population and military forces. In the event of mass casualty, shortage of standard antidotes may be anticipated, especially in the developing countries. In addition, the agriculture region with inadequate healthcare facilities is affected in many of the developing countries. Most of the unintentional or intentional OPC poisoning occurs commonly in these countries. The standard therapy which is not changed for many decades includes mainstay treatment of antimuscarinic agent atropine, an oxime and a benzodiazepine along with supportive measures. In addition, many different approaches and alternatives have been proposed in the literature [4] but none could practically replace the existing standard treatment. On the other hand, the

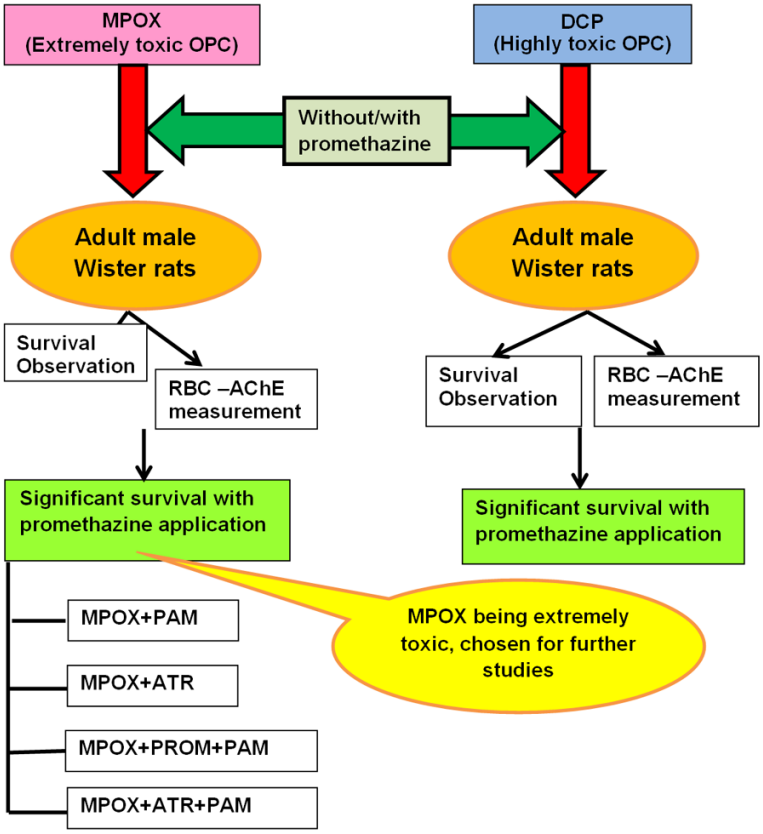
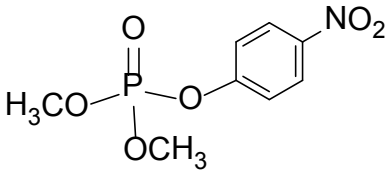
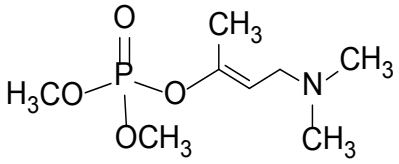
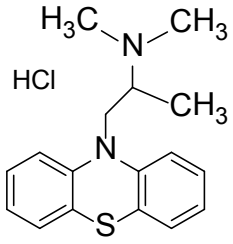
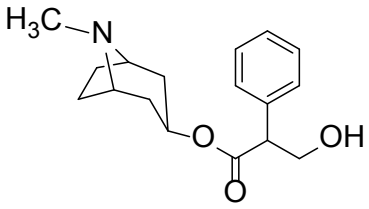
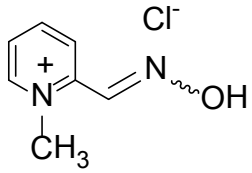


Figure 1. Schematic presentation of the work plan. Additional studies with MPOX includes survival studies, RBC-AChE, creatinine kinase and lactate dehydrogenase measurement.

increasing threat of nerve agents use against civilian population and forecasted increase in the use of OPCs in coming years calls for effective preparedness, and search for the effective, easily available, and cost effective/inexpensive antidotes.

The antihistamines as a tentative antidote for OPC poisoning have been scarcely studied. Some of the studies [5-13] and recent review by Ojha et al. [14] are noteworthy in this connection. In these studies, focus was mainly given on experimental studies conducted with old-generation antihistamine, e.g. diphenhydramine. Albeit, the conventional thinking of OPC toxicity is the inhibition of AChE, it is well established that many other non-cholinergic factors play a crucial role in mortality and morbidity. One of these factors is the stimulation of mast cell degranulation, possibly causing the release of histamine or histamine-like compounds that can precipitate the inflammatory processes [15, 16]. Accordingly, histamine released from mast cells binds to histamine H1 receptors which increases capillary permeability, initiate vasodilation and cause inflammatory responses [17]. For instance, Sarin, an OPC nerve agent has recently been reported to elevate histamine levels in the broncho-alveolar lavage of guinea pigs [18]. In addition, malathion metabolites were reported to induce histamine release from basophils and peritoneal mast cells [16]. Antihistamines have been described to act as anti-inflammatory agents by preventing histamine release from mast cells and/or stabilizing histamine receptors in an inactive conformation. Interestingly, neuro-inflammations with severe neuronal disorders by acute or chronic exposure of OPC have been widely documented in the literature [19, 20]. Hence, centrally acting old-generation antihistamines, e.g. PROM, may be useful in preventing the delayed deleterious effect of OPC poisoning, through its multi-pharmacological actions. The antihistamine PROM has been used for several decades, and its pharmacokinetics and toxicity are well documented and established. Also, it is supposed to be available in almost all the clinical settings. A potent anti-cholinergic (both muscarinic and nicotinic), antihistaminic, a centrally acting drug, an inhibitor of human α 7 nicotinic acetylcholine receptor, and an adrenergic blocker, this drug was considered as a proficient antidote for OPC poisoning. Moreover, the compound is easily and widely available, inexpensive and obtainable in large quantities in most of the hospitals and pharmacies in the event of mass casualty. In the present study, PROM is tested against paraoxon-methyl, (extremely toxic OPC; WHO class IA) and dicrotophos (highly toxic OPC; WHO class IB; **Figure 1**) [21]. MPOX is the most potent among AChE inhibiting OPCs, and about 70% as potent as the nerve agent sarin. The proposed study will provide an alternative to existing atropine therapy, especially in

Table 1. Chemical structure of the tested compounds

Compound	Structural formula	Empirical formula (Hill Notation)	Molecular weight
Paraoxon-methyl (O,O-Dimethyl O-(4-nitrophenyl) phosphate)		$C_8H_{10}NO_6P$	247.14
Dicrotophos 3-(dimethoxy phosphinyloxy)-N,N-dimethylcis-crotonamide		$C_8H_{16}NO_5P$	237.19
Promethazine 10-[2-(Dimethylamino) propyl]phenothiazine hydrochloride		$C_{17}H_{20}N_2SxHCl$	320.88
Atropine endo-(±)-α-(Hydroxymethyl)benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester		$C_{17}H_{23}NO_3$	289.37
Pralidoxime Pyridine-2-aldoxime methochloride		$C_7H_9N_2OxCl$	172.61

the event of shortages of supplies. In addition, the study will be a newer approach of using an antihistamine with multi pharmacological actions as antidote of OPC poisoning instead of prevailing anti-muscarinic agent.

Material and methods

Experimental animals

During the entire experiment, the "Guiding principles in the Care of and Use of Laboratory Animals" have been observed. Animals were handled, ethically treated, and humanly killed as per the rules and instructions of the Ethical Committee. All studies were performed with the approval of the Institutional Ethical Committee (A15/14). The original stock of Wistar rats was purchased from Harlan Laboratories (Harlan Laboratories, Oxon, England). The animals used in the present studies were bred at our own Animal Facility from the original stock. Adult male rats were housed in polypropylene cages (43 × 22.5 × 20.5 cm^3; six rats/cage) in climate- and access-controlled rooms (23±1°C; 50±4% humidity). The day/night cycle was 12 h/12 h. Food and water were available ad libitum. The food was purchased from Emirates Feed Factory (Abu Dhabi, UAE) which is a standard maintenance diet for rats.

Chemicals

Methyl Paraoxon (MPOX) and dicrotophos (DCP) stock solution (100 mM) was prepared in dry acetone. Working solution for i.p. application

Table 2. Percentage of mortality after treatment with MPOX (G1) and DCP (G3) alone and simultaneous application of PROM (G2 & G4). First row in each box shows percent mortality with standard error of mean. Second row is the 95% confidence interval of the mean. Data is derived from n=18 to 24 treated rats in 3-4 cycles of experiments

Groups	30 min.	60 min.	120 min.	180 min.	240 min.	1440 min.	2880 min.
G1: MPOX only	73±10 (46-100)	73±10 (46-100)	73±10 (46-100)	73±10 (46-100)	73±10 (46-100)	73±10 (46-100)	73±10 (46-100)
G2: MPOX+PROM	17±7 (0-38)	17±7 (0-38)	17±7 (0-38)	17±7 (0-38)	17±7 (0-38)	17±7 (0-38)	17±7 (0-38)
G3: DCP only	56±119 0-143	78±11 30-126	78±11 30-126	78±11 30-126	78±11 30-126	78±11 30-126	78±11 30-126
G4: DCP+PROM	11±6 0-35	17±10 0-58	17±10 0-58	17±10 0-58	22±6 0-46	22±6 0-46	22±6 0-46

Table 3. Percentage of mortality after treatment with different treatment regimens of MPOX and PROM, ATR, PAM. First row in each box shows percent mortality with standard error of mean. Second row is the 95% confidence interval of the mean. Data is derived from n=18 to 24 treated rats in 3-4 cycles of experiments

Groups	30 min.	60 min.	120 min.	180 min.	240 min.	1440 min.	2880 min.
G5: MPOX+PAM	29±24 (0-100)	29±24 (0-100)	42±22 (0-100)	42±22 (0-100)	42±22 (0-100)	42±22 (0-100)	42±22 (0-100)
G6: MPOX+ATR	13±8 (0-38)	13±8 (0-38)	13±8 (0-38)	13±8 (0-38)	13±8 (0-38)	13±8 (0-38)	13±8 (0-38)
G7: MPOX+PROM+PAM	9±5 (0-24)	9±5 (0-24)	9±5 (0-24)	9±5 (0-24)	9±5 (0-24)	9±5 (0-24)	9±5 (0-24)
G8: MPOX+ATR+PAM	39±15 (0-100)	50±10 (8-92)	50±10 (8-92)	50±10 (8-92)	50±10 (8-92)	50±10 (8-92)	50±10 (8-92)

was prepared ex tempore by diluting stock solution with saline. The other solutions were prepared before experiment. All the chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Chemical structures of the compounds used in the present study are provided in **Table 1**.

Choice of dosage and treatment

The acute dose (≈ LD_{75}) of MPOX and DCP and half of LD_{01}, of PAM was selected to administer to the animals. PAM, PROM and ATR dosage was selected according to previous studies [10, 22, 23]. Kan et al. [10] used 40 mg/kg body weight against soman (a nerve agent) poisoning. Since our study deals with a lesser toxic OPC than nerve agent, hence we selected a slightly reduced dose that is 30 mg/kg body weight. Sanderson [23] investigated the effect of i.p. administered atropine (17.4 mg/kg) given alone, or combined with oximes against different OPCs in rats and found promising survival results. We opted for 18 mg/kg body weight based on Sanderson work. A schematic plan of conducted experiments is shown in **Figure 1**.

Reference groups

Only MPOX exposure: 1.98 mg/kg average body weight diluted in 500 µl saline solution. Only DCP exposure: 14 mg/kg average body weight diluted in 500 µl saline solution. PAM: 35 mg/kg average body weight diluted in 500 µl saline solution. PROM: 30 mg/kg average body weight diluted in 500 µl saline solution. ATR: 18 mg/kg body weight diluted in 500 µl saline solution.

Experimental groups and exposure of compounds

There were eight groups of experimental rats. The experiments were repeated four times (3-4 cycles; 6 rats/group/cycle). The Ist and 3rd group was given MPOX and DCP i.p. alone respectively. Group 2 & 4 received MPOX and DCP injection and, thereafter (within one minute) i.p.

Table 4. Mean survival time in minutes over the period in different groups. All the treatment groups are statistically significantly improved in survival than no treatment group that is MPOX and DCP only

	Mean ± SEM	95% CI
MPOX only	623.75±236.26	160.68-1086.82
MPOX+PROM	2405.00±216.81	1980.06-2829.94
DCP only	670.00±278.45	124.24-1215.76
DCP+PROM	2265.00±271.34	1733.18-2796.82
MPOX+PAM	1347.500±287.81	783.39-1911.61
MPOX+ATR	2523.75±192.40	2164.65-2900.85
MPOX+PROM+PAM	2642.50±160.78	2327.36-2957.65
MPOX+PAM+ATR	1458.33±335.10	801.54-2115.12

All the treatment groups are statistically significantly improved in survival than no treatment group i.e. MPOX only. *statistically significant than G1 (MPOX).

Table 5. Pairwise comparison using Log-Rank (Mantel-Cox) test in Kaplan-Meir survival analysis

	MPOX	MPOX+PROM	DCP	DCP+PROM
MPOX	--	0.000	0.605	0.000
MPOX+PROM	0.000	--	0.000	0.032
DCP	0.605	0.000	--	0.000
DCP+PROM	0.000	.699	0.000	--

injection of PROM. Groups 5-8 received i.p. injections of PAM, PROM or ATR according to the groups mentioned below.

The animals were monitored for 48 hours and mortality was recorded at 30 min, 1, 2, 3, 4, 24 and 48 hours. There were 3 control groups with 6 rats each. Control groups received only the PROM, PAM and ATR respectively but no MPOX injections.

Test groups

Group 1 (G1): MPOX only. Group 2 (G2): MPOX+PROM. Group 3 (G3): DCP only. Group 4 (G4): DCP+PROM. Group 5 (G5): MPOX+PAM. Group 6 (G6): MPOX+ATR. Group 7 (G7): MPOX+PROM+PAM. Group 8 (G8): MPOX+ATR+PAM.

Control groups

Group 9 (G9): PROM ONLY. Group 10 (G10): PAM only. Group 11 (G11): ATR only.

RBC-AChE activity

The blood samples for RBC-AChE measurement was collected from the tail vein. The RBC-AChE activity was measured in diluted whole blood samples in the presence of the selective butyryl-cholinesterase inhibitor, ethoproprazine as described previously [24]. The values were normalized to the hemoglobin (Hb) content (determined as cyanmethemoglobin) and expressed as mU/µmol Hb.

Blood collection for biochemical tests

After forty eight hours of treatment, blood samples from rats were collected by decapitation, centrifuged at 3000 rpm for 10 minutes. Serum obtained was then stored frozen at -80°C. CK and LDH were performed by auto-analyzer, COBAS INTEGRA 400 PLUS from Roche Diagnostics, Germany.

Statistical analysis

Statistical analysis was performed on the mortality data of four cycles. Kaplan-Meir survival analysis was performed using SPSS 21.0 statistical software, SPSS Inc., Chicago, IL, USA. Kaplan-Meier survival analysis allows estimation of survival over time. The time starting from a defined point to the occurrence of a given event, for instance death is called as survival time. For each interval, survival probability is calculated as survivors divided by experimental individuals at risk. Experimental individual who have died, are not counted as "at risk". Non-parametric Manwhitney-U test was used for statistical significance and $P \leq 0.05$ was considered significant. SPSS 21.0 software package was used for all statistical evaluations.

Results

Mortality/survival analysis

Tables 2 and **3** show the percentage of animals died at different time points over the period of 48 hours in MPOX and DCP treatment and after application of PROM. The results observed clearly show that PROM significantly provided a protective effect in both extremely and highly toxic OPC intoxicated groups (**Tables 2** and **3**). The mean survival times estimated by Kaplan Meier survival analysis showed 2405.00± 216.81 minutes in MPOX+PROM treated group as compared to 623.75±236.26 in MPOX only group. Similarly, mean survival time in DCP+PROM treated group was 2265.00±271.34 minutes as compared to 670.00±278.45 min-

Table 6. Pairwise comparison using Log-Rank (Mantel-Cox) test in Kaplan-Meir survival analysis

	MPOX	MPOX+PROM	MPOX+PAM	MPOX+ATR	MPOX+PROM+PAM	MPOX+PAM+ATR
MPOX	---	0.000	0.046	0.000	0.000	.038
MPOX+PROM	0.000	--	0.008	0.686	0.388	.023
MPOX+PAM	0.046	0.008	--	0.003	0.001	.859
MPOX+ATR	0.000	0.686	0.003	--	0.640	.009
MPOX+PROM+PAM	0.000	0.388	0.001	0.640	--	.003
MPOX+PAM+ATR	0.038	0.023	0.859	0.009	0.003	--

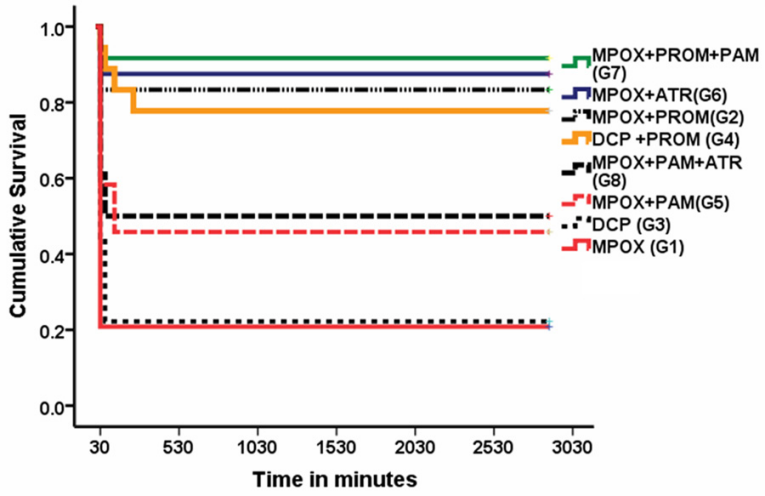


Figure 2. Kaplan-Meier survival plot: Survival is statistically significantly increased by all treatment groups. Legends are shown according to efficacy in plot. Higher protection was achieved when PROM and PAM is given together (G7; topmost line) followed by G6, G2, G4, G8 and G5. The standard application (PAM+ATR; G8) yielded less protection than other treatment groups.

utes in DCP treated group (**Table 4**). The outcome was statistically significant (**Table 5**). **Table 4** shows the survival analysis with MPOX, PAM and ATR (G5 TO G8). The mortality of control rats that had only received PAM, PROM and ATR, was 0%. Kaplan Meier survival curves are shown in **Figure 2**. The maximum survival was achieved in Group 7 (MPOX+*PROM*+PAM) followed by Group 6 (MPOX+ATR) and then Group 2 (MPOX+*PROM*). The standard combination, namely, MPOX+ATR+PAM (Group 8) ranked fourth. Based on the standard treatment protocol, it is evident that Group 8 is less effective than PROM administered regimes (**Table 4** and **Figure 2**). The notion that "administration of PAM along with ATR is not worthy" is also noted in the results of survival times in minutes after treatment. Interestingly, the *P*-values calculated for the pair-wise comparison (**Tables 5** and **6**) revealed that administration of PROM instead of ATR produced higher survival times which are also statistically significant from control group.

RBC-AChE activities

The enzyme activities (% of baseline) in different groups are shown in **Figures 3** and **4**. The enzyme activities were measured in the survived animals (n=3-10) at 30 minutes, 24 and 48 hours after treatment. The mortality was high in MPOX and DCTP treatment groups, assuming less than 10% RBC-AChE activity which is not incorporated in the data, and hence, could not be measured practically. The RBC-AChE at 30 minutes was almost the same in all groups except the group which received MPOX+PROM, and was found to be with values that were less than the values observed for animals in Group 1 (*P*-value=0.020). Conversely, it was one of the most promising protection groups in terms of survival. Similarly, MPOX+PAM+ATR group showed increased reactivation of RBC-AChE at all-time points but number of survivor was less than the PROM administered groups. Overall, reactivation of RBC-AChE was progressively higher at 24 hours and 48 hours, even in the groups where PAM was not injected. A ranking of the reactivators according to their ability to increase enzyme activity is difficult since the data available are, for obvious reasons, from surviving animals only and may thus be biased.

Serum creatinine and LDH

Creatinine and LDH levels in serum are shown in **Table 7**. Though variations may be noted in

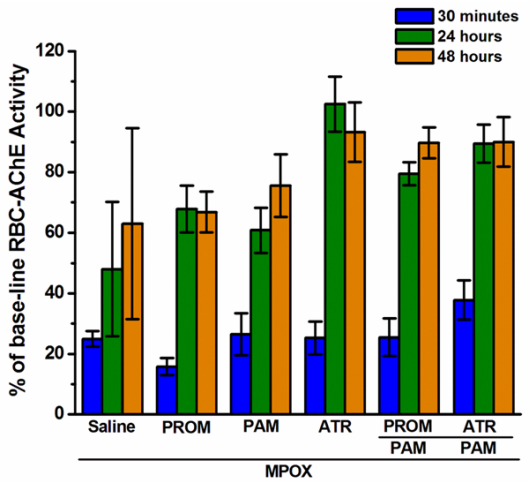


Figure 3. Percentage of RBC-AChE activity at three-time points in MPOX treated groups.

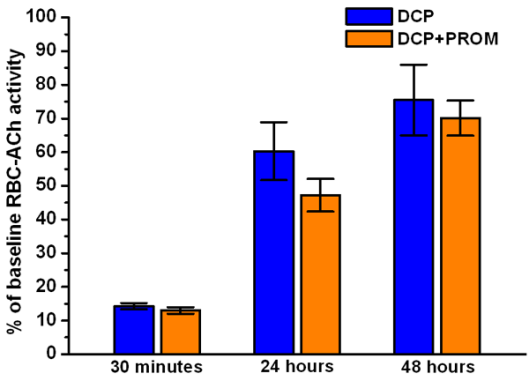


Figure 4. Percentage of RBC-AChE activity at three-time points in dicrotophos treated groups.

the values but statistically no significance was found when compared with MPOX treatment group. The results suggest that administration of PROM failed to produce profound effect leading to cardiac toxicity. On the other hand, when PROM was administered alone (control group), it produced markedly but statistically not significant increase in CK level. The only statistical significance was noted in LDH level of MPOX+PROM+PAM group where it was found to be decreased when compared with the MPOX treated group.

Discussion

The treatment of poisonings produced by OPC-AChE inhibitors has remained unchanged for many decades with the muscarinic antagonist atropine (ATR) being used as a primary antidote. Meanwhile, application of OPC pesticides has been increased and anticipated to increase multifold in future. Likewise, in the event of mass casualty, shortage of medicine may be anticipated. Keeping all such circumstances, and based on the reality that the standard ATR is being mainly anti-muscarinic, the present study was designed to investigate the protective potency of PROM, an old-generation antihistamine with well-known centrally acting pharmacological profile which includes anti-muscarinic and anti-nicotinic effects. Two structurally (**Table 1**) and functionally (potentially) different OPCs were used to assess the protective effect of PROM.

The results the current study showed promising protective effect (**Tables 3** and **4**) of PROM against MPOX which is an extremely toxic and dicrotophos, a highly toxic OPC according to WHO, classification of pesticides. There was no statistical significance between MPOX+ATR and MPOX+PROM treatment groups. Noteworthy, the protection observed by the standard clinical application (MPOX+PAM+ATR) was less than by the group treated with MPOX+PROM. The latter finding reflects the concern raised by many researchers and clinicians regarding the use of PAM with ATR [25]. There is only one conference report by Kan et al. [10] who showed promising protection by PROM against OPC Sarin in rats. However, the first study on the use of antihistamine for OPC poisoning was reported in 1963 by Welch and Coon [5]. They concluded that pretreatment with chlorcyclizine significantly reduced the mortality induced by parathion, a parent compound of paraoxon, in mice. Previous antidotal efficacy studies of old-generation antihistamines were mainly focused on diphenhydramine [7-9, 12, 13, 26, 27]. Notably, Gupta et al. [28] reported a clinical case study, where cyproheptadine was tested against an OPC poisoned patient and found effective. Our studies with old-generation antihistamine, PROM, also showed good antidotal efficacy against MPOX and DCP which is in concurrence to earlier studies. Among the various probabilities of effectiveness, PROM is a centrally acting potent anticholinergic compound with both muscarinic and nicotinic components. It is well-known that OPC poisoning produces both muscarinic and nicotinic symptoms whereas the conventional antidotes usually include only antimuscarinic compounds. There

Table 7. Creatinine kinase and Lactate dehydrogenase level in serum of control and treated groups

Groups	Creatinine kinase (U/L) Mean ± SD (*P* value)	Lactate dehydrogenase (IU/L) Mean ± SD (*P* value)
MPOX only	8007.0±1831.0	1031.3±394.0
MPOX+PROM	5736.0±1439.7 (0.071)	674.7±199.5 (0.071)
MPOX+PAM	8524.6±2319.0 (0.456)	975.4±325.5 (0.297)
MPOX+ATR	7842.7±1973.1 (0.796)	811.0±282.2 (0.439)
MPOX+PROM+PAM	7844.0±1183.0 (1.000)	651.3±107.8 (0.020)
MPOX+ATR+PAM	6834.7±471.5 (0.289)	906.7±819.6 (0.289)
PROM only	10054.8±1262.4 (0.121)	996.3±240.1 (0.796)
PAM only	7920.2±1154.9 (0.881)	786.4±369.5 (0.101)
ATR only	6917.3±1079.9 (0.606)	563.2±193.6 (0.071)
Saline control	6319.2±937.4 (0.327)	994.8±363.0 (0.221)

are accumulating arguments that support the usefulness to include anti-nicotinic compounds for a better protection [9]. Besides, it has been observed that early death due to OPC poisoning is considered to be centrally mediated [9]. Also, respiratory failure is a manifestation of acute OPC poisoning [29]. Thus, it may be implicated that the improved survival in the current study by using PROM may be due to its multi-pharmacological and centrally mediated action. The study does not provide clear evidence of role of PROM in the reactivation process of RBC-AChE, though the inhibition of RBC-AChE was almost similar to non-PROM treated group or even less. Since animals survived even at more than 90% of inhibition, a stage where animals do dot survives, it may be presumed that there me be little enough un-noticeable spontaneous reactivation of AChE that resulted in survival of animals. But the multiple pharmacological action of PROM is indeed a factor for the survival whether by prompting spontaneous reactivation, centrally mediated anti-nicotinic or anti-muscarinic action or by altering the detoxifying enzyme as found by Welch and Coon [5]. Petroianu et al. [22, 30, 31] investigated tiapride for acute paraoxon poisoning against rats and found very improved survival, though the RBC-AChE level was less than paraoxon alone treatment, and concluded that peripheral enzyme activity and mortality are not strongly correlated. Based on the evidences, it may be speculated that merely RBC-AChE is not essential for survival, rather some other mechanisms also play role in the survival after OPC poisoning.

It is noteworthy to mention that many other symptoms and factors are associated with OPC poisoning which may become a co-lethal and mortality factor but by large they are ignored. For instance, OPC intoxication also increases the release of biogenic amines including histamine in addition to many pro-inflammatory cytokines which causes neuro-inflammation [17, 19]. Cowan et al. [32] concluded that non-cholinergic mechanism of OP poisoning often includes anaphylactic shock which is prompted by autocoids such as histamine. Importantly, activation and degranulation of mast cells and basophils lead to the release of histamine, cytokines and other mediators into the extracellular environment and to the development of anaphylaxis. For instance, soman (a nerve agent OPC) has been reported to induce dose-dependently mast cell degranulation in rats and calcium dependent release of histamine from rat peritoneal mast cells. Sarin, (another nerve agent) vapor elevates histamine levels in Broncho alveolar lavage of guinea pigs [33]. Similarly, malathion metabolites have been reported to induce histamine release from basophils and peritoneal mast cells [34]. Furthermore, antihistamines are anti-inflammatory agents that act by preventing histamine release from mast cells and/or stabilizing histamine receptors in an inactive conformation. Therefore, it is suggested that sufferers of OPC poisoning may benefit from the potential anti-inflammatory properties of the antihistamines [35]. An additional promising feature of PROM which is recently reported is its potent antagonist property at various ion-gated channels

including human α-7 nicotinic acetylcholine receptors (α-7 nAChRs) [36]. Similarly, HI-6, an oxime with higher efficacy than other existing oxime-derived compounds used in OPC poisoning, was reported to have anti-nicotinic properties [37, 38]. Moreover, a potent protection was reported against sarin and tabun OPC nerve agents by the use of nicotinic antagonists [39]. Interestingly, α-7 nicotinic receptors have been linked to a wide variety of brain functions including pathological conditions such as Parkinson's and Alzheimer's disease which are reported to be delayed effects of OPC poisoning. In conclusion, the multiple pharmacological action of PROM including its anti-muscarinic, anti-nicotinic, antihistaminic, α-7 nAChR antagonistic effects along with its centrally mediated actions have produced the better protection in rat model which warrants further investigation as well. Additional investigations using different doses of PROM with various OPCs are also required to further evaluate the substantial risk/benefits of the clinical use of PROM.

Limitations

PROM has been reported to produce QT- prolongation effect. On the other hand, OPCs also cause bradycardia or tachycardia depending upon the type of the respective OPC, necessitating further investigations for the evaluation of its risks/benefits. However, it may be noted that high atropinaztion in OPC poisoning also causes serious adverse effect [40] and still ATR is the mainstream anti-muscarinic agent for OPC poisoning. In spite of the aforementioned side effect of PROM, the compound is widely available even as an OTC compound in different developing countries. In short, death/survival is still at present the gold standard one has to look at. Also, efficacy can only be defined by an increase in survival/reduction in mortality when looking at antidotal therapy for an agent with high acute toxicity. Any effect whether demonstrated in vitro or in vivo is meaningless unless it is translated to an increase/decrease in survival.

Conclusion

Promethazine, an old-generation antihistamine, has been found to be an effective antidotal agent for methyl-paraoxon and dicrotophos acute toxicity in Wistar rats. The observed efficacy was higher than that of ATR. The protective effect obtained in the present study was different from improving RBC-AChE, a marker enzyme. So, multi-pharmacological actions of PROM may be contributing to its enhanced protection. Additionally, PROM was not able to affect CK and LDH, indicating that there are no apparent signs of cardiac toxicity. Since toxicological profile of different OPC greatly varies, further investigations should be undertaken with other groups of OPCs.

Acknowledgements

Support to BS was provided by a UAEU Program for Advanced Research (UPAR) Grant (#31M-126) and Faculty Grant (#NP-15-34), UAE University.

Disclosure of conflict of interest

None.

Address correspondence to: Bassem Sadek, Department of Pharmacology, College of Medicine and Health Sciences, United Arab Emirates University, PO Box 17666, Al Ain, UAE. Tel: +971 3 7137 512; Fax +971 3 7672 033; E-mail: bassem.sadek@uaeu.ac.ae

References

[1] Macilwain C. Study proves Iraq used nerve gas. Nature 1993; 363: 3.

[2] Solberg Y and Belkin M. The role of excitotoxicity in organophosphorous nerve agents central poisoning. Trends Pharmacol Sci 1997; 18: 183-185.

[3] Nozaki H, Aikawa N, Fujishima S, Suzuki M, Shinozawa Y, Hori S and Nogawa S. A case of VX poisoning and the difference from sarin. Lancet 1995; 346: 698-699.

[4] Nurulain SM. Different approaches to acute organophosphorus poison treatment. JPMA 2012; 62: 712-717.

[5] Welch RM and Coon JM. Studies on the effect of chlorcyclizine and other drugs on the toxicity of several organophosphate anticholinesterases. J Pharmacol Exp Ther 1964; 143: 192-198.

[6] Newball HH, Donlon MA, Procell LR, Helgeson EA and Franz DR. Organophosphate-induced histamine release from mast cells. J Pharmacol Exp Ther 1986; 238: 839-845.

[7] Faris GA and Mohammad FK. Prevention and treatment of dichlorvos-induced toxicosis in mice by diphenhydramine. Vet Hum Toxicol 1997; 39: 22-25.

[8] Bird SB, Gaspari RJ, Lee WJ and Dickson EW. Diphenhydramine as a protective agent in a rat model of acute, lethal organophosphate poisoning. Acad Emerg Med 2002; 9: 1369-1372.
[9] Bird SB, Gaspari RJ and Dickson EW. Early death due to severe organophosphate poisoning is a centrally mediated process. Acad Emerg Med 2003; 10: 295–298.
[10] Kan RK, Tompkins CP, Kniffin DM and Hamilton TA. Promethazine as a novel prophylaxis and treatment for nerve agent poisoning. http://www.dtic.mil/dtic/tr/fulltext/u2/a505934.pdf (Last accessed on 11.7.15).
[11] Mousa YJ. Effect of chlorpheniramine on acute dichlorvos poisoning in chicks. Iraqi J Vet Sci 2009; 23: 35-43.
[12] Mohammad F, Faris G and Shindala M. Comparative antidotal effects of diphenhydramine and atropine against dichlorvos-induced acute toxicosis in rats. Vet Arhiv 2002; 72: 19-28.
[13] Mohammad FK, al-Kassim NA and Abdul-Latif AR. Effect of diphenhydramine on organophosphorus insecticide toxicity in mice. Toxicology 1989; 58: 91-95.
[14] Ojha S, Sharma C and Nurulain SM. Antihistamines: Promising antidotes of organophosphorus poisoning. Voj Zdrav Listy 2014; 83: 1-7.
[15] Rodgers K and Xiong S. Contribution of mast cell mediators to alterations in macrophage function after malathion administration. Fundam Appl Toxicol 1996; 33: 100-108.
[16] Rodgers K and Xiong S. Contributions of inflammatory mast cell mediators to alterations in macrophage function after malathion administration. Int J Immunopharmacol 1997; 19: 149-156.
[17] Banks CN and Lein PJ. A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation. Neurotoxicology 2012; 33: 575-584.
[18] Levy A, Chapman S, Cohen G, Raveh L, Rabinovitz I, Manistersky E, Kapon Y, Allon N and Gilat E. Protection and inflammatory markers following exposure of guinea pigs to sarin vapour: comparative efficacy of three oximes. J Appl Toxicol 2004; 24: 501-504.
[19] Nurulain SM, Adeghate E, Sheikh A, Yasin J, Kamal MA, Sharma C, Adem A and Ojha S. Sub-chronic exposure of non-observable adverse effect dose of terbufos sulfone: neuroinflammation in diabetic and non-diabetic rats. CNS Neurol Disord Drug Targets 2014; 13: 1397-405.
[20] Wani WY, Sunkaria A and Sharma DR, Kandimalla RJL, Kaushal A, Gerace E, Chiarugi A, Gill KD. Caspase inhibition augments Dichlorvos-induced dopaminergic neuronal cell death by increasing ROS production and PARP1 activation. Neuroscience 2014; 258: 1-15.
[21] The WHO recommended classification of pesticides by hazard and guidelines to classification [Internet]. 2004 [cited 2015 Apr 28]. Available from: http://www.who.int/ipcs/publications/pesticides_hazard_rev_3.pdf.
[22] Petroianu GA, Hasan MY, Nurulain SM, Arafat K, Sheen R and Nagelkerke N. Comparison of two pre-exposure treatment regimens in acute organophosphate (paraoxon) poisoning in rats: tiapride vs. pyridostigmine. Toxicol Appl Pharmacol 2007; 219: 235-240.
[23] Sanderson DM. Treatment of poisoning by anticholinesterase insecticides in the rat. J Pharm Pharmacol 1961; 13: 435-442.
[24] Worek F, Mast U, Kiderlen D, Diepold C and Eyer P. Improved determination of acetylcholinesterase activity in human whole blood. Clin Chim Acta 1999; 288: 73-90.
[25] Peter JV, Moran JL and Graham P. Oxime therapy and outcomes in human organophosphate poisoning: an evaluation using meta-analytic techniques. Crit Care Med 2006; 34: 502-510.
[26] Mohammad F, Mousa Y and Al-Zubaidy M. Assessment of diphenhydramine effects against acute poisoning induced by the organophosphate insecticide dichlorvos in chicks. Hum Vet Med 2012; 4: 6-13.
[27] Yavuz Y, Yurumez Y, Ciftci IH, Sahin O, Saglam H and Buyukokuroglu M. Effect of diphenhydramine on myocardial injury caused by organophosphate poisoning. Clin Toxicol (Phila) 2008; 46: 67-70.
[28] Gupta V, Karnik ND, Deshpande R and Patil MA. Linezolid-induced serotonin syndrome. BMJ Case Rep 2013; 2013.
[29] Eddleston M, Mohamed F, Davies JOJ, Eyer P and Worek F, Sheriff MHR and Buckley NA. Respiratory failure in acute organophosphorus pesticide self-poisoning. QJM 2006; 99: 513-522.
[30] Petroianu GA, Hasan MY, Nurulain SM, Arafat K, Sheen R, Saleh A and Schmitt A. Protective drugs in acute large-dose exposure to organophosphates: a comparison of metoclopramide and tiapride with pralidoxime in rats. Anesth Analg 2005; 100: 382-386.
[31] Petroianu GA, Hasan MY, Nurulain SM, Arafat K, Shafiullah M and Sheen R. Tiapride pretreatment in acute exposure to paraoxon: comparison of effects of administration at different points-in-time in rats. Mol Cell Biochem 2006; 285: 79-86.
[32] Cowan FM, Shih TM, Lenz DE, Madsen JM and Broomfield CA. Hypothesis for synergistic toxicity of organophosphorus poisoning-induced cholinergic crisis and anaphylactoid reactions. J Appl Toxicol 1996; 16: 25-33.

[33] Doebler JA, Shih TM and Anthony A. Quantitative cytophotometric analyses of mesenteric mast cell granulation in acute soman intoxicated rats. Experientia 1985; 41: 1457-1458.
[34] Xiong S and Rodgers K. Effects of malathion metabolites on degranulation of and mediator release by human and rat basophilic cells. J Toxicol Environ Health 1997; 51: 159-175.
[35] Kamanyire R and Karalliedde L. Organophosphate toxicity and occupational exposure. Occup Med Oxf Engl 2004; 54: 69-75.
[36] Sadek B, Khanian SS, Ashoor A, Prytkova T, Ghattas MA, Atatreh N, Nurulain SM, Yang K-HS, Howarth FC and Oz M. Effects of antihistamines on the function of human α7-nicotinic acetylcholine receptors. Eur J Pharmacol 2015; 746: 308-316.
[37] Soukup O, Kumar UK, Proska J, Bratova L, Adem A, Jun D, Fusek J, Kuca K and Tobin G. The effect of oxime reactivators on muscarinic receptors: functional and binding examinations. Environ Toxicol Pharmacol 2011; 31: 364-370.
[38] Soukup O, Jun D, Tobin G and Kuca K. The summary on non-reactivation cholinergic properties of oxime reactivators: the interaction with muscarinic and nicotinic receptors. Arch Toxicol 2013; 87: 711-719.
[39] Turner SR, Chad JE, Price M, Timperley CM, Bird M, Green AC and Tattersall JEH. Protection against nerve agent poisoning by a noncompetitive nicotinic antagonist. Toxicol Lett 2011; 206: 105-111.
[40] Economacos G and Kanakis J. A case of hypersensitivity to atropine. Anesth Analgésie Réanimation 1981; 38: 748.