CLINICAL & BASIC RESEARCH

In Vitro Cysteine Reactivates Organophosphate Insecticide Dichlorvos-Inhibited Human Cholinesterases

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الإضافة للسيستين يعيد تنشيط أنزيم كولينستراز البشري التي يثبطها مبيد الحشرات العضوية الفوسفاتية الديكلوروفوس

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ABSTRACT: ***Objectives:*** Organophosphate (OP) pesticides inhibit both red blood cell (RBC) and plasma cholinesterases (ChEs). Oximes, especially pralidoxime (2-PAM), are widely used as antidotes to treat OP poisoning. In addition, N-acetylcysteine (NAC) is sometimes used as an adjuvant antidote. The current study aimed to assess the feasibility of using NAC as a single therapeutic agent for OP poisoning in comparison to *in vitro* 2-PAM. ***Methods:*** This study was carried out at the Razi Drug Research Center of Iran University of Medical Sciences, Tehran, Iran, between April and September 2014. A total of 22 healthy human subjects were recruited and 8 mL citrated blood samples were drawn from each subject. Dichlorvos-inhibited blood samples were separately exposed to low and high doses (final concentrations of 300 and 600 µmol.L^{-1}, respectively) of 2-PAM, NAC and cysteine. Plasma and RBCs were then separated by centrifugation and their ChE activity was measured using spectrophotometry. ***Results:*** Although cysteine—and not NAC—increased the ChE activity of both plasma and RBCs over those of dichlorvos, it did not increase them over those of a high dose of 2-PAM. ***Conclusion:*** These results suggest that the direct reactions of 2-PAM and cysteine with dichlorvos and the reactivation of phosphorylated ChEs occurr via an associative stepwise addition-elimination process. High therapeutic blood concentrations of cysteine are needed for the elevation of ChE activity in plasma and RBCs; however, both this agent and NAC may still be effective in the reactivation of plasma and RBC ChEs.

Keywords: Organophosphate Poisoning; Antidotes; Cysteine; N-Acetylcysteine; Pralidoxime Compounds; Cholinesterases.

الملخص: **الهدف:** مبيدات الفوسفات العضوي هي مواد من صنع الإنسان وهي تثبط انزيم الكولينستراز في كل من خلايا الدم الحمراء (كرات الدم الحمراء) و البلازما. وتستخدم الأوكسيمات، و خصوصا البراليدوكسيم بصورة شائعة، كترياق لعلاج التسمم بمبيدات الفوسفات العضوي. وبالأضافة الي ذلك فقد تستخدم مادة ال ن-أسيتيل سيستين كعامل علاجي احدي للتسمم بمبيدات الفوسفات العضوي ومقارنتها مع مادة البراليدوكسيم في المختبر. **الطريقة:** أجريت هذه الدراسة في مركز الرازي لبحوث المخدرات، جامعة ايران للعلوم الطبية، طهران، إيران، بين أبريل وسبتمبر 2014. في هذه الدراسة تم سحب ثمانية ميلي ليتر عينات دم معالج بمادة السيترات من 22 شخصا سليما. تم تعريض عينات الدم المثبطة بمادة الديكلوروفوس بشكل منفصل لجرعات منخفضة ثم مرتفعة من البراليدوكسيم و ال ن-أسيتيل سيستين والسيستين (بتركيزات نهائية مقدارها 300 ميكرومولار 600 ميكرومولار علي التوالي). بعد ذلك تم فصل البلازما وكرات الدم الحمراء من العينات بواسطة الطرد المركزي تلاها القياس الطيفي لنشاط أنزيم الكولينستراز بهم. **النتائج:** أظهرت النتائج أنه على الرغم من السيستين (وليس ال ن-أسيتيل سيستين) أدى الي زيادة كبيرة في نشاط انزيم الكولينستراز في كل من البلازما وكرات الدم الحمراء تغلبت على تثبيط مادة الديكلوروفوس، ولكنها ليست بنفس النسبة التي تحدثها تلك الجرعة العالية من البراليدوكسيم. **الخلاصة:** تشير نتائج الدراسة الحالية الي أن التفاعلات المباشرة بين كل من البراليدوكسيم والسيستين مع مادة الديكلوروفوس تؤدي ألي تنشيط انزيم الكولينستراز المفسفر، بطريقة تصاعدية تعتمد على الأضافة والحذف. وبالرغم من الحاجة ألي وجود تركيزات عالية من السيستين في الدم لكي تزيد من نشاط انزيم الكولينستراز الا ان هذه الماده ومادة ال ن-أسيتيل سيستين قد يكون لهما دور فعال في اعادة تنشيط انزيم الكولينستراز في كل من البلازما وكرات الدم الحمراء.

الكلمات المفتاحية: التسمم بمركبات الفوسفات العضوي؛ ترياق؛ السيستين؛ ن-أسيتيل سيستين؛ مركبات البراليدوكسيم؛ انزيم الكولينستراز.

ADVANCES IN KNOWLEDGE

- *The results of the present study showed that, although high concentrations of cysteine increased dichlorvos-inhibited acetyl- and pseudocholinesterase activities, this agent was less efficient than pralidoxime in the reactivation of human cholinesterases.*

- *In addition, the study illustrated that cysteine directly reacts with and inactivates dichlorvos at low plasma concentrations, while it replaces serine in the dichlorvos-cholinesterase complex at a very high plasma concentration and regenerates inhibited cholinesterases as well as having a direct reaction with dichlorvos.*

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- These findings indicate that the effectiveness of cysteine in dichlorvos-induced poisoning depends on both dichlorvos and cysteine plasma concentrations.

Application to Patient Care
- According to the findings of this study, the efficacy of cysteine in the treatment of cases of organophosphate poisoning may depend on maintaining high therapeutic concentrations of cysteine.

ORGANOPHOSPHATE (OP) PESTICIDES ARE MAN-made phosphoric acid esters that inhibit and inactivate both true (i.e. acetylcholinesterase [AChE]) and pseudo- (i.e. butyrylcholinesterase [BuChE]) cholinesterases (ChEs) by a nucleophilic reaction in which the associative two-step addition-elimination reaction with the serine hydroxyl group at their active sites results in phosphorylated and inactivated enzymes.[1,2] AChE is primarily found in nervous tissue and erythrocytes; erythrocytic ChE activity more accurately reflects nervous tissue AChE activity than BuChE, which is primarily produced in the liver and appears in plasma, with its activity varying with a number of disorders including liver disease.[3,4] The irreversible inactivation of ChEs by the dealkylation of the OP moiety of the OP-ChE complex is known as ageing, in which the phosphorus-oxygen bond of the OP-ChE complex strengthens.[5] After ageing occurs, even oximes are ineffective in reactivating the enzyme, and the only means of restoring ChE activity is via the lengthy process of synthesizing a new AChE enzyme.[2]

At present, two main antidotes exist for the treatment of OP poisoning: atropine and oximes, particularly pralidoxime (2-PAM) in the latter group.[2] However, oximes are not always useful in the management of OP poisoning.[6–8] Moreover, the phosphorylated oxime complex is a strong inhibitor of ChEs and oximes themselves can inhibit AChEs.[9] Thus, the introduction of new, more efficient and less toxic antidotes to OP poisoning seems necessary. N-acetylcysteine (NAC) is a prodrug of cysteine and a mucolytic agent with potent antioxidant and free radical scavenging properties; it is currently used to treat acetaminophen poisoning and as an adjuvant antidote for OP poisoning.[10,11] Other medical uses of NAC include the treatment of radiocontrast-induced nephropathy, cyclophosphamide-induced haemorrhagic cystitis and a number of psychiatric disorders.[12–14] Furthermore, NAC has significant anti-viral activity against the *influenza A* viruses.[15] The present study aimed to assess the feasibility of cysteine administration as a single therapeutic agent for the reactivation of both AChE and BuChE in comparison with *in vitro* 2-PAM administration.

Methods

This study was carried out at the Razi Drug Research Center, Iran University of Medical Sciences, Tehran, Iran, between April and September 2014. A total of 22 healthy human subjects were recruited to participate in the study. Each participant was requested to complete a questionnaire to determine their health status, use of medications, occupational history and exposure to pesticides. Subjects with a history of diabetes mellitus, hypertension, liver disease, anaemia, malnutrition, cancer or other chronic illnesses and those who smoked cigarettes, used alcohol or drugs or had a history of radiotherapy were excluded from the study.

The following chemicals and solutions were purchased: 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB), N-acetylcysteine, cysteine, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium chloride and tripotassium citrate monohydrate (Merck KGaA, Darmstadt, Germany); quinidine sulfate dihydrate, acetylthiocholine iodide and S-butyryl-thiocholine iodide (Sigma-Aldrich Corp., St. Louis, Missouri, USA); dichlorvos of 98% weight/weight purity (Gyah Corp., Karaj, Iran); and 2-PAM methyl-sulfate (Laboratoires SERB, Paris, France). Stock solutions of dichlorvos, 2-PAM, NAC and L-cysteine were each separately prepared in a 0.9% weight/volume (w/v) solution of sodium chloride in distilled water (i.e. normal saline) to produce final concentrations of 21.0 μg.mL⁻¹ (95 μmol.L⁻¹), 14.9 mg.mL⁻¹ (60 mmol.L⁻¹), 9.80 mg.mL⁻¹ (60 mmol.L⁻¹) and 7.27 mg.mL⁻¹ (60 mmol.L⁻¹). All stock solutions were stored in the dark at 4 °C.

An 8-mL blood sample was obtained from each subject and immediately citrated with a 3.8% w/v tripotassium citrate solution to prevent clot formation.[16] Each sample was then divided into 1 mL *aliquots*. The first *aliquot* was considered as a control and placed in a water bath at 37 °C for 80 minutes. The second *aliquot* was exposed to dichlorvos at a final concentration of 0.95 μmol.L⁻¹ in a 37 °C water bath for 80 minutes.[17] The remaining *aliquots* were exposed to dichlorvos at a final concentration of 0.95 μmol.L⁻¹ in a water bath at 37 °C for 20 minutes, followed by

exposure to low and high concentrations of 2-PAM, L-cysteine and NAC separately in a water bath at 37 °C for 60 minutes. In total, 10 μL of the dichlorvos, 2-PAM, NAC and cysteine solutions were added to 1 mL of blood to produce final concentrations of 0.21 μg.mL^{-1} (0.95 μmol.L^{-1}), 149 μg.mL^{-1} (600 μmol.L^{-1}), 98 μg.mL^{-1} (600 μmol.L^{-1}) and 72.7 μg.mL^{-1} (600 μmol.L^{-1}), representing the high doses of the drugs. An additional 5 μL of the 2-PAM, NAC and L-cysteine solutions were added to 1 mL of blood to produce final concentrations of 74.5 μg.mL^{-1} (300 μmol.L^{-1}), 49 μg.mL^{-1} (300 μmol.L^{-1}) and 36.3 μg.mL^{-1} (300 μmol.L^{-1}), representing the low doses of the drugs.

The plasma and red blood cells (RBCs) of each blood sample were then separated by centrifugation at 5,000 revolutions per minute for 10 minutes. Each plasma sample was diluted 100 times with distilled water and the diluted sample was used to determine BuChE activity. Each volume of the RBC sample was first washed three times with three volumes of normal saline and then haemolyzed and diluted 60 times with distilled water to sufficiently dilute thiol-containing (i.e. cysteine and NAC) and oxime-containing (i.e. 2-PAM) compounds and prevent reactions with DTNB. The haemolyzed diluted sample (haemolysate) was used to determine AChE activity. Ellman's kinetic method was used to determine the BuChE and AChE activities of diluted samples at 410 and 440 nm, respectively.[18]

In order to measure BuChE activity, 50 μL of the diluted plasma sample was added to 150 μL of a reagent containing 0.423 mmol.L^{-1} of DTNB in 0.1 mol.L^{-1} of a phosphate buffer with a pH of 7.6 as well as 50 μL of substrate (10 mmol.L^{-1} of butyrylthiocholine iodide in distilled water) in each well of a 48-well plate and marked as a test well. All components of the test well were included in five blank wells, except for the sample which was replaced by an equivolume of normal saline. Initial and final absorbances of the test and blank wells were then recorded at 410 nm over a 5-minute period. Test well absorbances were subtracted from the average absorbance of the blank wells and divided by 0.0216 as the product of the time period and the molar attenuation coefficient of the 5-thio-2-nitrobenzoate anion at 410 nm (0.0136 L.μmol^{-1}.cm^{-1}) and light path length (0.318 cm) and multiplied by the dilution factor (100 x 5 = 500).

Subsequently, AChE activity was measured using 10 μL of the haemolysate sample added to 300 μL of a reagent containing 0.27 mmol.L^{-1} of DTNB and 20 μmol.L^{-1} of quinidine sulfate in 0.1 mol.L^{-1} of a phosphate buffer at a pH of 7.6 and preincubated for 5 minutes at 37 °C. This procedure was followed by the addition of 10 μL of substrate (100 mmol.L^{-1} of acetylthiocholine iodide in distilled water) in each well of a 48-well plate and marked as a test well. As before, five blank wells contained all components of the test well, except for the sample, which was replaced by an equivolume of normal saline. The initial and final absorbances of the test and blank wells were recorded during a 5-minute period at 440 nm. The absorbances of the test wells were subtracted from the average absorbance of the blank wells and divided by 0.023 as the product of the time period and the molar attenuation coefficient of the 5-thio-2-nitrobenzoate anion at 440 nm (0.01434 L.μmol^{-1}.cm^{-1}) and light path length (0.32 cm) and multiplied by the dilution factor (60 x 32 = 1,920). The visible absorbances of samples were measured using a multi-mode microplate reader (Synergy™ HT Microplate Reader, BioTek Instruments Inc., Winooski, Vermont, USA).

All BuChE and AChE activities were expressed in units per L (U.L^{-1}). The activity of each sample was divided by that of the respective control (normalisation) and represented as a percentage. A one-sample t-test was used to analyse the statistical differences between the percentages of BuChE and AChE activities in the dichlorvos and control groups, for which the latter was deemed to have 100% ChE activity. Differences between percentages of BuChE and AChE activities in the experimental groups were analysed using a one-way analysis of variance test followed by a Scheffe *post-hoc* test. The Statistical Package for the Social Sciences (SPSS), Version 23.0 (IBM Corp., Armonk, New York, USA) was used for all statistical analyses. A *P* value of <0.050 was considered statistically significant. Graphs were produced using SigmaPlot software, Version 12.0 (Systat Software Inc., San Jose, California, USA).

This study was approved by the Ethics Committee of the Mazandaran University of Medical Sciences, Sari, Mazandaran, Iran (#1072). All procedures were performed in accordance with the ethical standards of the revised Declaration of Helsinki of 2008. All participants signed an informed consent form before being included in the study.

Results

Dichlorvos significantly reduced BuChE activity in comparison to the control group (37.8% ± 2.5%; *P* <0.010). Both low and high doses of 2-PAM (55.0% ± 3.5% and 72.0% ± 3.4%, respectively) significantly increased BuChE activity in comparison to the dichlorvos group (*P* <0.010 and <0.001, respectively). Low concentrations of NAC and cysteine (38.8% ± 2.0% and 35.5% ± 2.7%, respectively) did not significantly
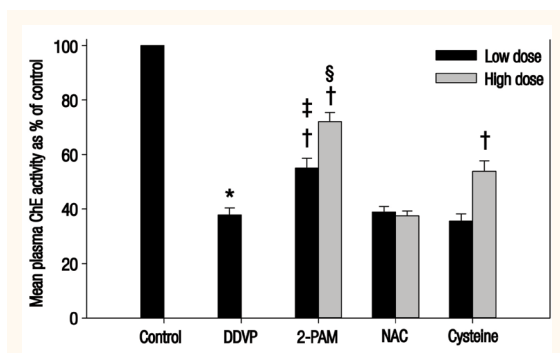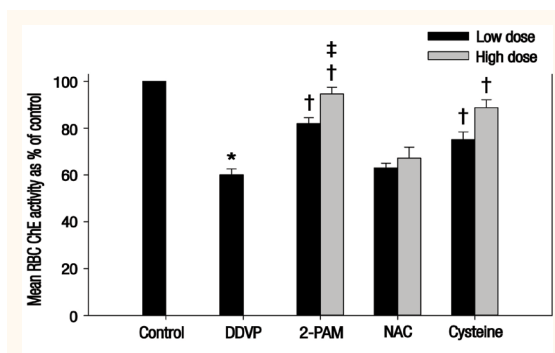
Figure 1: Chart showing mean plasma cholinesterase activity in different experimental groups as a measure of human cholinesterase reactivation in the treatment of organophosphide poisoning (N = 22). The dichlorvos group was compared with the control group while all other groups were compared with the dichlorvos group.

ChE = cholinesterase; DDVP = dichlorvos; 2-PAM = pralidoxime; NAC = N-acetylcysteine.

*Statistically significant difference in comparison to the control group (P <0.010). †Statistically significant difference in comparison to the dichlorvos group (P <0.010). ‡Statistically significant difference in comparison to a low dose of cysteine (P <0.010). §Statistically significant difference in comparison to a low dose of 2-PAM (P <0.010).

Figure 2: Chart showing mean red blood cell cholinesterase activity in different experimental groups as a measure of human cholinesterase reactivation in the treatment of organophosphide poisoning (N = 22). The dichlorvos group was compared with the control group while all other groups were compared with the dichlorvos group.

RBC = red blood cell; ChE = cholinesterase; DDVP = dichlorvos; 2-PAM = pralidoxime; NAC = N-acetylcysteine.

*Statistically significant difference in comparison to the control group (P <0.010). †Statistically significant difference in comparison to the dichlorvos group (P <0.010). ‡Statistically significant difference in comparison to a low dose of cysteine (P <0.010).

increase BuChE activity over dichlorvos (P >0.050 each). A high dose of NAC (37.5% ± 1.7%) also did not significantly increase BuChE activity in comparison to dichlorvos (P >0.050). However, a high concentration of cysteine (53.8% ± 3.9%) significantly increased BuChE activity over that of dichlorvos (P <0.010). A high concentration of 2-PAM significantly increased BuChE activity in comparison to a low concentration (P <0.010). Moreover, a low dose of 2-PAM significantly increased BuChE activity in comparison to a low dose of cysteine (P <0.010) [Figure 1].

AChE activity was significantly reduced in the dichlorvos group compared to the control group (60.0% ± 2.5%; P <0.010), although to a lesser extent than for BuChE activity. Both low and high doses of 2-PAM (81.9% ± 2.4% and 94.6% ± 2.8%, respectively) significantly increased AChE activity over that of dichlorvos (P <0.010 each). Low and high concentrations of NAC (63.0% ± 2.0% and 67.1% ± 4.7%, respectively) did not significantly increase AChE activity in comparison to dichlorvos (P >0.050 each). Low and high concentrations of cysteine (75.0% ± 3.3% and 88.7% ± 3.4%, respectively) significantly increased AChE activity compared to the dichlorvos group (P <0.010 and <0.010, respectively). There was no significant difference between low and high concentrations of 2-PAM or low concentrations of 2-PAM and cysteine in terms of AChE activity (P >0.050 each). There was also no significant difference between high concentrations of 2-PAM and cysteine with regards to AChE activity. However, a high dose of 2-PAM significantly increased AChE activity over that of a low cysteine dose (P <0.010) [Figure 2].

Discussion

Previous research has indicated that NAC replenishes glutathione storage and has antioxidant and free radical scavenging properties.[19] It is a prodrug which is enzymatically metabolised (i.e. deacetylated) by the liver into its active metabolite cysteine and serves as a cysteine donor.[20] The thiol group of cysteine is reactive toward xenobiotics and seems to be responsible for its beneficial effects in poisoning cases.[21] In the present study, the reactivation effect of equimolar concentrations of 2-PAM, NAC and cysteine on dichlorvos-induced BuChE and AChE inhibition was investigated. The results demonstrated that dichlorvos inhibited BuChE activity to a greater degree than AChE activity; this finding is in agreement with a statement by Jafari *et al.* indicating that AChE is less sensitive to OPs than BuChE.[22] Ríos *et al.* have previously shown that while 2-PAM concentrations as low as 66 μmol.L⁻¹ are sufficient to prevent deaths in most *in vivo* cases of OP poisoning, concentrations of up to 700 μmol.L⁻¹ are much more effective.[23] In the present study, samples were treated with both low (300 μmol.L⁻¹) and high (600 μmol.L⁻¹) concentrations of 2-PAM in order to compare ChE reactivation effects; the results showed that a high concentration of 2-PAM increased BuChE but not AChE activity in comparison to a low concentration. These findings confirm that ChE reactivation in OP poisoning is dependent on the plasma concentrations of 2-PAM.[23]

The concentrations of 2-PAM, NAC and cysteine in the current study were selected based on their therapeutic plasma concentrations in humans.[23,24] The

standard intravenous regimen of NAC—150 mg/kg[-1] for 15 minutes followed by 50 mg/kg-1 for four hours and 100 mg/kg[-1] over the next 16 hours—produces initial NAC plasma concentrations ranging from 304–875 μg.mL^{-1}.[24] Concentrations subsequently fall rapidly and reach a relatively constant level of approximately 35 μg.mL^{-1} after 12 hours until the infusion is stopped.[24] In comparison to dichlorvos, concentrations of NAC in the present study did not significantly increase BuChE and AChE activities; however, both BuChE and AChE activities significantly increased with concentrations of cysteine. This finding indicates that cysteine, the active metabolite of NAC, is responsible for the reactivation of ChEs. The absence of a metabolising organ—the liver—in the present *in vitro* study may explain why NAC did not elevate BuChE and AChE activities in comparison to cysteine.[20] Thus, in clinical situations in which liver function is normal, NAC treatment may still be effective. The results of the present work indicate that therapeutic plasma concentrations of cysteine (or, potentially, NAC) for the treatment of OP poisoning strongly depends on OP plasma concentrations.

The pK_a of nucleophiles has been shown to play an important role in their reaction with OPs, in that more basic nucleophiles with higher pK_a values show a lower reactivity.[2,25] The pK_a values of the 2-PAM oxime and cysteine thiol groups are 7.8 and 8.3, respectively, which reflect a favourable range.[2,26] In addition, according to the Henderson-Hasselbalch equation, the 2-PAM oxime and cysteine thiol groups are 28.48% and 11.18% ionised at a pH of 7.4, respectively.[2] Thus, it is expected that, in addition to 2-PAM, cysteine could also react with the OP-ChE complex and displace OP with a nucleophilic attack, resulting in the reactivation of ChEs. With a pK_a of 9.5, NAC is less reactive to nucleophiles than cysteine.[27] For a nucleophilic attack of the cysteine thiol group to the phosphorus-oxygen bond of the OP-ChE complex, the phosphorus-oxygen bond between the serine hydroxyl oxygen atom from ChE and the phosphorous atom of OP should be cleaved and a phosphorus-sulfur bond formed between the cysteine thiol sulfur atom and the phosphorus atom of OP. Cysteine is a weak nucleophile and the phosphorus-sulfur bond is much weaker than a phosphorus-oxygen bond and therefore easier to break; however, the concentration of the reactants, namely cysteine and OP, seems to play a critical role in this reaction.[28,29] On the basis of collision theory, a higher concentration of reactants increases the probability of effective collisions, consequently resulting in the formation of a product.[30] However, to be effective, collisions should occur in a particular molecular orientation.

In the present study, concentrations of dichlorvos and cysteine were 0.95 μmol.L^{-1} and 300 μmol.L^{-1} (low dose) and 600 μmol.L^{-1} (high dose), respectively. In clinical cases of OP poisoning, the plasma concentration of OP is very high due to the high intake.[31] Thus, large amounts of NAC and even 2-PAM should be administered intravenously to be effective. Some researchers have reported that although intravenous NAC reduced hospitalisation time among OP-poisoned individuals, it unfortunately did not increase ChE activity.[11] One explanation for this outcome may be related to the low plasma concentration of NAC in comparison to the high plasma concentration of OP. Another explanation may be related to the direct reaction of NAC—or, more accurately, cysteine-with OPs which results in more polar and may be more excretable product.[2] A similar result has been observed with the administration of 2-PAM to OP-poisoned individuals, in that ChE reactivation seems to depend on the plasma concentrations of 2-PAM and OP, with high concentrations of 2-PAM necessary for the adequate reactivation of OP-inhibited human RBC ChEs.[23] This phenomenon explains why oximes are not always useful in the management of OP poisoning.[6–8]

Figure 3 illustrates the proposed mechanism of nucleophilic attack of 2-PAM and cysteine to dichlorvos, in which the reactions of 2-PAM and cysteine with dichlorvos proceed through an associative stepwise addition-elimination mechanism.[2] In both of these reactions, the 2,2-dichlorovinylate anion (as a leaving group) is separated from a pentacoordinate phosphorene intermediate and leaves a polar product which seems to be more excretable than dichlorvos. The 2,2-dichlorovinylate anion then gains a hydrogen ion and is converted to 2,2-dichlorovinyl alcohol which in turn undergoes a spontaneous rearrangement and is converted to dichloroacetaldehyde, a metabolite of dichlorvos.[2] One of the detoxification mechanisms of NAC in acetaminophen poisoning is its direct reaction with N-acetyl-p-benzoquinone imine, the toxic metabolite of acetaminophen.[19] Thus, NAC may still act as an antidote in OP poisoning due to its direct reaction and neutralisation properties. On this basis, Figure 4 depicts another proposed mechanism for the reactivation of OP-inhibited ChEs by 2-PAM and cysteine wherein the reactions of 2-PAM and cysteine with OP-inhibited ChEs also proceed via an associative stepwise addition-elimination process. Similarly, the serine anion of ChE as a leaving group is separated from a pentacoordinate phosphorane intermediate in both of these reactions.

The pK_a of the leaving group has also been shown to play an important role in the reaction of nucleophiles, as more acidic leaving groups with low pK_a values are
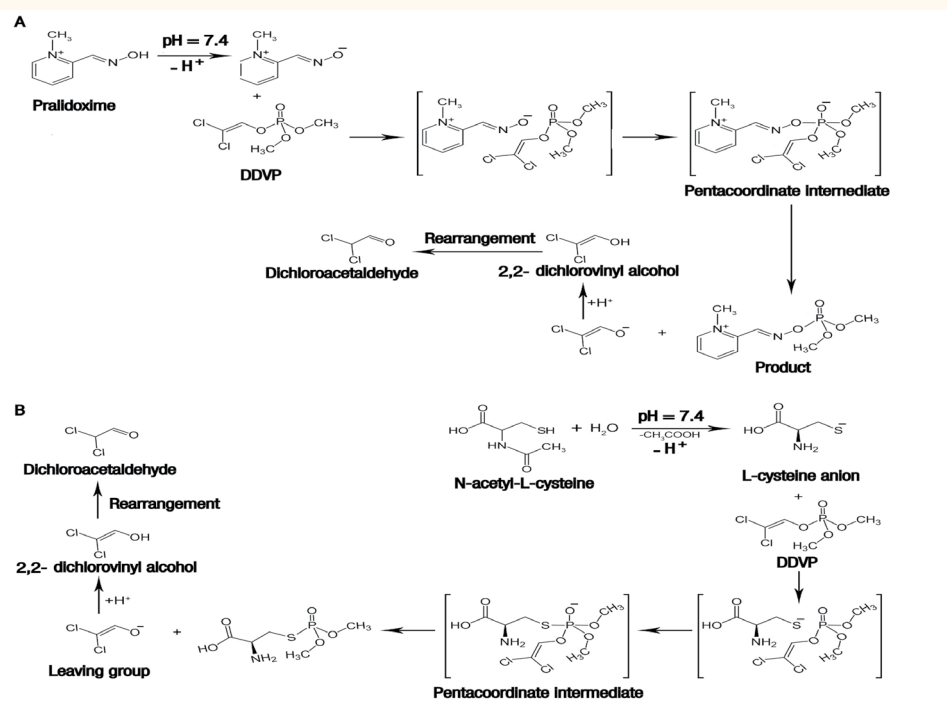
Figure 3: Diagram showing a proposed nucleophilic mechanism for the reaction of **(A)** pralidoxime and **(B)** N-acetylcysteine with dichlorvos.

CH₃/H₃C = methyl group; N = nitrogen; OH = hydroxide; H = hydrogen; Cl = chlorine; O = oxygen; P = phosphorus; DDVP = dichlorvos; NH₂ = amine group; S = sulfur.

Figure 3A reproduced with permission from Shetab-Boushehri SV, Shetab-Boushehri SF, Abdollahi M. Possible role of Mg2+ ion in the reaction of organophosphate (dichlorvos) with serine.[2]
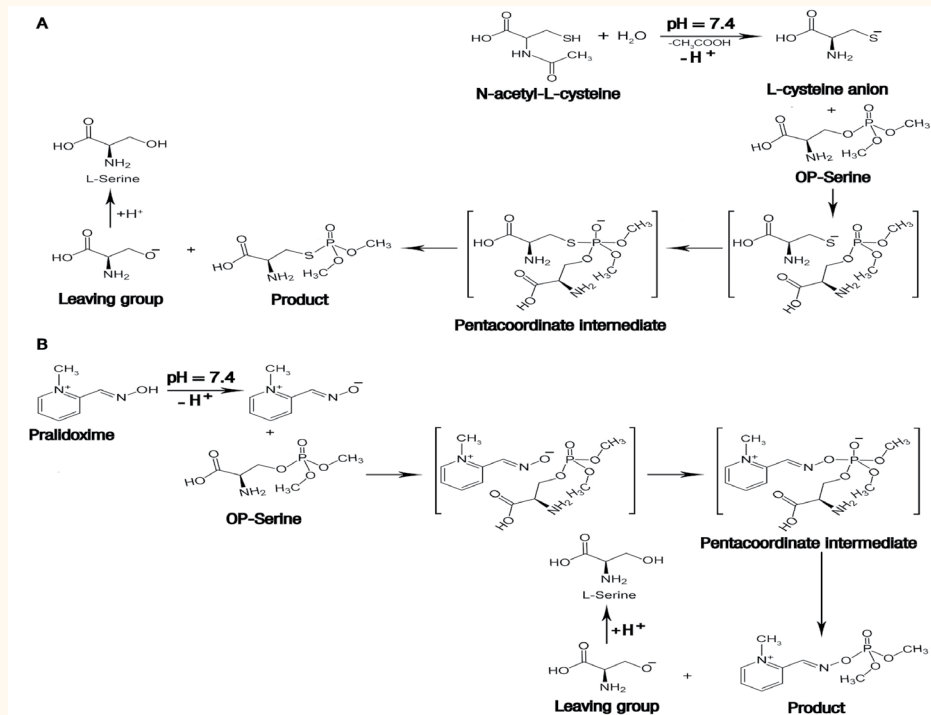
Figure 4: Diagram showing a proposed nucleophilic mechanism for the reaction of **(A)** pralidoxime and **(B)** N-acetylcysteine with the dichlorvos-cholinesterase complex. For simplification, only the serine moiety of cholinesterase is shown.

CH₃/H₃C = methyl group; N = nitrogen; OH = hydroxide; H = hydrogen; O = oxygen; NH₂ = amine group; P = phosphorus; OP = organophosphate; S = sulfur; HN = amine; SH = thiol; H₂O = water.

more easily separated from OPs.[25] This could explain why certain OPs more rapidly age ChEs in comparison to others.[5] In the current study, the displacement of serine as a leaving group with a pK_a of 13.0 by 2-PAM and cysteine as nucleophiles with pK_as of 7.8 and 8.3, respectively, seems thermodynamically unfavourable;[2,26] nevertheless, clinical evidence as well as the results of the present study show that although 2-PAM has a lower pK_a than serine, it displaces the serine moiety in the OP-ChE complex which results in reactivated ChEs.[3,4,8,9] Cysteine, although a weaker nucleophile, seems to follow this mechanism but at much higher plasma concentrations. As in the case of direct reactions of 2-PAM and cysteine with dichlorvos, the displacement of the serine moiety of the OP-AChE complex by 2-PAM and cysteine seems to be dose-dependent. Although cysteine is a weak nucleophile, the current study demonstrates that it nevertheless displaces the serine moiety in the OP-ChE complex at high concentrations.

The authors suggest the administration of a maximum tolerable intravenous dose of NAC for the treatment of OP poisoning; however, extensive animal studies are needed to confirm this suggestion and NAC should be considered a supportive antidote until the safety and efficacy of this approach is approved. Considering its potential for metabolising into cysteine, NAC could be an alternative therapeutic agent in OP poisoning. A comparative animal study on the beneficial effects of NAC and cysteine on the reactivation of ChEs in OP poisoning is therefore proposed for future research.

Conclusion

The results of the present study suggest that the direct reactions of 2-PAM and cysteine with dichlorvos, and the subsequent reactivation of phosphorylated ChEs, proceed through an associative stepwise addition-elimination process. In addition, the findings indicate that cysteine directly reacts with OPs at low concentrations while, at very high concentrations, it replaces serine in the pentacoordinate intermediate and regenerates inhibited ChEs as well as directly reacting with dichlorvos. Moreover, it is possible that higher doses of NAC in a clinical context may also have the same effect; however, further studies are required to confirm this theory.

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