N-acetylcysteine in Acute Organophosphorus Pesticide Poisoning: A Randomized, Clinical Trial

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Abstract: Organophosphorus poisoning is a major global health problem with hundreds of thousands of deaths each year. Research interest in N-acetylcysteine has grown among increasing evidence of the role of oxidative stress in organophosphorus poisoning. We aimed to assess the safety and efficacy of *N*-acetylcysteine as an adjuvant treatment in patients with acute organophosphorus poisoning. This was a randomized, controlled, parallel-group trial on 30 patients suffering from acute organophosphorus poisoning, who were admitted to the Poison Control Center of Tanta University Emergency Hospital, Tanta, Egypt, between April and September 2014. Interventions included oral *N*-acetylcysteine (600 mg three times daily for 3 days) as an added treatment to the conventional measures *versus* only the conventional treatment. Outcome measures included mortality, total dose of atropine administered, duration of hospitalization and the need for ICU admission and/or mechanical ventilation. A total of 46 patients were screened and 30 were randomized. No significant difference was found between both groups regarding demographic characteristics and the nature or severity of baseline clinical manifestations. No major adverse effects to *N*-acetyl-cysteine therapy were reported. Malondialdehyde significantly decreased and reduced glutathione significantly increased only in the NAC-treated patients. The patients on NAC therapy required less atropine doses than those who received only the conventional treatment; however, the length of hospital stay showed no significant difference between both groups. The study concluded that the use of *N*-acetylcysteine as an added treatment was apparently safe, and it reduced atropine requirements in patients with acute organophosphorus pesticide poisoning.

Organophosphorus (OP) insecticides are among the most important pesticides, and poisoning induced by them represents a major global health problem with hundreds of thousands of deaths each year, mostly in developing countries [1].

Organophosphorus pesticides inhibit esterase enzymes, especially acetylcholinesterase, which results in accumulation of acetylcholine at cholinergic synapses and overstimulation of cholinergic receptors of the autonomic nervous system, central nervous system (CNS) and neuromuscular junctions [2].

Acute toxicity produces a range of clinical manifestations, known as the acute cholinergic crisis. Depending on the type of receptors and their location, the clinical features may include muscarinic (bronchospasm, bronchorrhea, miosis, lachrymation, urination, diarrhoea, hypotension, bradycardia, vomiting and salivation), nicotinic (fasciculations, muscle weakness, paralysis, mydriasis, tachycardia, hypertension and sweating) and CNS manifestations (confusion, agitation, seizures, coma and respiratory failure) [3].

The leading cause of death in OP poisoning is respiratory failure, which may result from centrally or peripherally mediated mechanisms. It may occur either during the acute cholinergic crisis or shortly after an apparent recovery phase [4].

Diagnosis is made on the basis of history of acute exposure, development of characteristic clinical features and quantification of acetylcholinesterase or butyrylcholinesterase activity.

Although it has no relation to the severity of clinical toxicity, butyrylcholinesterase is particularly useful because of its sensitivity and wide availability [5,6].

Different mechanisms have been described for OP toxicity. One of the most important suggested mechanisms is induction of oxidative stress and reduction of antioxidant enzyme activity, which has been reported in a number of human and animal studies [7–9]. Organophosphates-induced formation of reactive oxygen species (ROS) may result from metabolism by cytochrome P450s, which catalyse oxidation by the addition of one atom of molecular oxygen into the OP molecule by an electron transport pathway resulting in generation of ROS [10].

In addition to classic treatments, the use of new classes of oximes [11] or drugs with antioxidant effects might therefore be a promising therapeutic option to dampen the increased ROS and renovate the antioxidant enzyme system. Scavengers of ROS were mostly studied in experimental OP poisoning. Both animal and *in vitro* research work used different antioxidants such as *N*-acetylcysteine (NAC) [7], ubiquinone [12] and vitamin C [13]. However, to the best of our knowledge, only one human study [14] used NAC as an antioxidant against acute OP poisoning.

N-acetylcysteine is a widely available, inexpensive drug, which has long been used for the treatment of patients with potentially lethal acetaminophen overdoses. It has a role in limiting hepatic or renal injury caused by xenobiotics that cause glutathione depletion and free radical formation including

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acetaminophen, carbon tetrachloride, chloroform and radiographic contrast agents [15].

N-acetylcysteine is a scavenger of ROS, but it acts primarily as a glutathione precursor, which is essential for the body's antioxidant defences by neutralizing reactive oxygen and nitrogen species through both direct and indirect scavenging. Furthermore, NAC might improve blood flow in the microvasculature and enhance oxygen delivery and utilization by different organs, such as the brain, heart and kidney [16].

In recent years, NAC has attracted increasing concern as a therapeutic agent for a number of xenobiotics-induced toxicities where oxidative stress is thought to contribute [17–19]. However, NAC has not been adequately studied for any of these xenobiotics in human beings to definitively recommend it as a therapeutic intervention.

Research interest in NAC has grown among increasing evidence of the role of oxidative stress in acute OP poisoning. *N*-acetylcysteine has been shown to attenuate free radical injury, recover organ dysfunction and improve survival rates in animal models intoxicated with OP [20,21].

It is prudent to examine inexpensive antidotes operating through synergistic mechanisms that are not dependent upon acetylcholinesterase reactivation or cholinergic receptor blockade. Hence, this study was conducted to assess the safety and efficacy of NAC as an adjuvant in the management of patients of acute OP pesticide poisoning.

Patients and Methods

Patients. This study was a randomized, controlled, parallel-group trial conducted on patients suffering from acute OP poisoning who were admitted within 12 hr after exposure to the Poison Control Center of Tanta University Emergency Hospital, Tanta, Egypt, between April and September 2014. The study was approved by the Research Ethics Committee of the Faculty of Medicine, Tanta University. An informed written consent was obtained from each patient (or his attending relatives if unable to do so) after receiving detailed information about the study (Research ethics committee approval number: 2457/03/14, Universal trial number: U1111-1155-5041, Trial ID on Australian New Zealand Clinical Trials Registry: ACTRN12614000407695).

Eligibility criteria. Acute symptomatic OP-intoxicated patients (male or female; aged 18 years or older; by any route of exposure), with no history of diabetes mellitus, cardiovascular, respiratory, renal and hepatic failure, and no medical management for OP poisoning in any medical centre before admission, were included in this study. The diagnosis in all cases was established on the basis of (i) the typical cholinergic toxidrome due to and following shortly after a single exposure to OP pesticide and (ii) a reliable identification of the compound based on the container brought by patient attendants and a subsequent confirmation by estimation of butyrylcholinesterase activity in all patients. Pregnant and lactating women, patients with ingestion or exposure to other substances in addition to the OP and those presenting more than 12 hr of exposure to the OP compound were excluded from this study. Neither analysis of the content of the containers brought nor measurement of the OP or its metabolites in the patient urine was performed.

Methods. A total of 46 patients were screened, with 16 patients excluded due to failure to meet eligibility criteria (five hepatic, three pregnant, two cardiac, six below 18 years old). The study volunteers (30 patients) were randomly allocated into two equal groups A (conventional treatment plus NAC) and B (conventional treatment) using the sequentially numbered, opaque sealed envelopes method [22]. The envelopes were impermeable to intense light, and the allocation sequence was concealed from the physician enrolling and assessing participants. To prevent subversion of the allocation sequence, the name and hospital admission number of the participant were written on the envelope. Carbon paper transferred the information onto the allocation card inside the envelope. Corresponding envelopes were opened only after the enrolled participants completed all baseline assessments, and it was time to allocate the intervention. The study participants, health care providers and data analysts were kept blinded to the allocation.

All patients received the conventional treatment, which included all or some of the following as indicated: patient resuscitation, decontamination, and atropine and obidoxime administration. Patients presenting within 2 hr of OP ingestion were subjected to gastric lavage, and all patients with oral exposure were given a single (50 mg) dose of activated charcoal. Any contaminated material was discarded, and dermal decontamination was carried out using soap and water, if necessary. Atropine (each ampoule contains 1 mg of atropine in 1 ml) was given as bolus doses of 2–5 mg IV and repeated every 10–15 min. until dryness of bronchial secretions, and then, atropine injections were given intermittently to patients as needed. Toxogonin® (each ampoule contains 0.25 g of obidoxime chloride in 1 ml, produced by Merck, Darmstadt, Germany) was administered as a loading dose of 250 mg bolus IV, followed by 250 mg, and repeated every 8 hr until at least 12 hr after atropine was no longer required [6]. In addition to the conventional treatment, group A received Acetylcisteine® (each effervescent instant sachet contains 200 mg NAC, produced by SEDICO Pharmaceuticals Co., Egypt, 6 October) orally, in a dose of 600 mg three times daily for 3 days. This dose of NAC has been reported to reduce oxidative stress and to improve antioxidant status in several clinical studies [23–25].

Patients were monitored with a detailed documentation of any adverse effect due to drug therapy. Group B received placebo in a similar order. The placebo contained sugar, and it was matched to the study drug for colour, consistency and size, but not for taste or smell.

All patients were subjected to full history taking (including age, gender, occupation, level of education, circumstances of poisoning, route of exposure, time interval between exposure and beginning of treatment and history of medical diseases) and complete physical examination (including level of consciousness by Glasgow coma scale, regular monitoring of vital signs and general clinical examination). Arterial blood samples were obtained from each patient for blood gas analysis, whereas venous samples were used for estimation of the biochemical profile, butyrylcholinesterase activity and oxidative stress biomarkers including malondialdehyde (MDA) [26] and reduced glutathione (GSH) [27].

All the patients were prospectively monitored by qualified physicians with regular measurement of their vitals and oxygen saturation via a bedside monitor, and they were followed up until discharge from the hospital. Adverse reactions to NAC were recorded and the severity of symptoms and signs of acute OP poisoning were graded according to Minton and Murray [28] and Bey *et al.* [29] into mild (fatigue, headache, blurred vision, dizziness, nausea, vomiting, excessive sweating, salivation, abdominal pain and chest tightness), moderate (symptoms of mild poisoning plus muscular fasciculation, weakness, inability to walk, chest crepitations and miosis) and severe (symptoms of moderate poisoning plus unconsciousness, flaccid paralysis, respiratory distress, cyanosis and marked miosis with loss of pupil reflexes).

Outcome measures. This study was a pilot phase II clinical trial to reveal the safety and efficacy of NAC as an added treatment in acute OP poisoning. The primary outcome was mortality, whereas secondary outcome measures included the length of hospital stay, the received total dose of atropine assessed at the time of discharge and the need of ICU admission and/or mechanical ventilation.

Statistics. The data were analysed according to intention to treat approach involving all patients who were randomly assigned. The primary objective of this study was to compare the incidence of mortality (Yes/No) in the study treatment groups. Secondary continuous outcome, as length of hospital stay and total dose of atropine required, was analysed using the Mann–Whitney test. Baseline data of study participants were collected and summarized in the form of mean ± S.D. for continuous outcome and in the form of frequency (relative frequency) for dichotomous outcome. Also, the data were analysed using the t-test or the Mann–Whitney U-test based on normal distribution assumption for continuous data and Fisher's exact test for categorical variables to check for imbalance between treatment arms. The *p*-values of 0.05 or less were considered to be statistically significant. Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) for Windows, version 15.0 (SPSS, Chicago, IL, USA).

Results

Table 1 shows baseline demographic and clinical characteristics of the study groups. The groups were homogeneous regarding age and gender. The patients were classified based on the severity of clinical manifestations. The study groups included comparable numbers of mild and moderate cases, but there was only one severe case included in group A. No patient complained of seizures. No significant difference was found between both groups regarding the nature or severity of baseline clinical manifestations. No major adverse effects to NAC were reported. Vomiting was not significantly different among patients who received NAC compared to those who did not receive NAC therapy. In only few cases, the OP involved was identified as chlorpyrifos or malathion. However, most of the cases could not be identified due to lack of information from the patients and their attendants.

The differences between serum butyrylcholinesterase activity before and after treatment within each study group were

Table 1.
Baseline demographic and clinical characteristics.

	Group A (n = 15) NAC + Standard care	Group B (n = 15) Standard care
Age (years)	39 (24)	35 (19)
Gender (male)	11 (73.3)	13 (86.7)
Severity		
Mild	9 (60)	9 (60)
Moderate	5 (33.3)	6 (40)
Severe	1 (6.7)	0 (0)
Mode of poisoning		
Accidental	12	13
Suicidal	3	2
Vomiting	15 (100)	13 (86.7)
Diarrhoea	9 (60)	9 (60)
Sweating	6 (40)	8 (58)
Bronchospasm	1 (6.7)	1 (6.7)
Abdominal colic	10 (66.7)	7 (46.7)
Bradycardia	13 (86.7)	12 (80)
Fasciculation	4 (26.7)	2 (13.3)
Coma	1 (6.7)	0 (0)
Delay time (hr)	3.13 (1.76)	3.53 (1.91)

Data are medians (interquartile range), means (S.D.) or numbers (%).

significant. Statistically significant differences were noticed between both groups in serum levels of each of the oxidative stress biomarkers MDA and GSH. Administration of NAC therapy to patients of group A was associated with a significant reduction of MDA but a significant increase in GSH when the mean level of each biomarker was compared before and after NAC therapy (table 2).

Neither mortality nor the need for of admission and/or mechanical ventilation was recorded in either group. The patients on NAC therapy required significantly less atropine doses than those who received standard management alone without NAC; however, the length of hospital stay showed no significant difference between both groups (table 3).

Discussion

In this randomized, controlled trial, the baseline characteristics showed no significant difference between the study groups. No major adverse effects to NAC therapy were reported. Malondialdehyde significantly decreased and reduced glutathione significantly increased only in the NAC-treated patients. Neither mortality nor the need of ICU admission and/or mechanical ventilation was recorded in either group. The patients on NAC therapy required less atropine doses, but the length of hospital stay showed no significant difference between both groups.

The most frequent adverse effects reported in the scientific literature for NAC includes vomiting and anaphylactoid reactions [15]. The current study reported no major adverse effects (e.g. anaphylactoid reactions) among patients given NAC treatment. Regarding vomiting, the study did not find any significant difference between patients who received NAC and those who did not receive NAC therapy. Because no controlled studies have compared intravenous with oral NAC, the oral route was preferred for more patient convenience. As this is a new indication for NAC therapy, the researchers were keen to use the lowest effective antioxidant dose [23–25] that could be tolerated by the patients.

The present study revealed marked reduction of serum butyrylcholinesterase activity on admission in each of the studied groups, but no significant difference between both groups was noticed. Comparable studies [30,31] reported that cholinesterase activities were considerably decreased in both acute and chronic OP intoxications. In the present study, butyrylcholinesterase activity increased significantly in either group after treatment – a logical finding that was reported also by others [14]. Although it was statistically insignificant, the overall increase of butyrylcholinesterase activity was slightly higher in the NAC-treated group compared to group B. Treatment with NAC was not associated with a considerable effect on butyrylcholinesterase activity. This might be due to using oximes in both groups, which played the major role in cholinesterase enzyme reactivation.

Serum MDA levels significantly decreased in group A patients, who received NAC treatment, but significantly increased in group B patients, who were given only conventional treatment. Many studies had shown that OP poisoning

Table 2.

Serum butyrylcholinesterase activity, serum malondialdehyde (MDA) and blood reduced glutathione (GSH) levels.

	Groups	On admission (mean ± S.D.)	After treatment (mean ± S.D.)	*p*-Value	*p*-Value (A−B)
Butyrylcholinesterase	Group A (n = 15)	2429.4 ± 1601	6067.13 ± 2162.28	0.001	0.768
(U/l)	Group B (n = 15)	3163.6 ± 1000.12	6604.33 ± 2282.65	0.001	
MDA (nmol/ml)	Group A (n = 15)	5.82 ± 2.49	2.78 ± 1.82	0.001	0.001
	Group B (n = 15)	3.09 ± 2.84	5.75 ± 2.13	0.005	
GSH (mg/dl)	Group A (n = 15)	0.49 ± 0.16	2.15 ± 0.49	0.001	0.002
	Group B (n = 15)	0.39 ± 0.24	0.41 ± 0.25	0.728	

Table 3.

Outcome measures.

	Group A (n = 15) NAC + Standard care	Group B (n = 15) Standard care	*p*-Value
Total atropine dose (mg)	5 (4)	11 (2)	0.003
Hospital stay duration (days)	3 (1)	2 (1)	0.143

induces the generation of ROS, thus increasing lipid peroxidation and production of MDA. Mashali *et al.* [32] reported significantly increased MDA serum levels in OP-intoxicated patients compared to healthy controls. In animal studies, Yurumez *et al.* [33] reported that treatment with NAC significantly decreased MDA levels in OP-intoxicated mice.

After NAC therapy, blood levels of GSH in group A patients increased significantly, whereas group B patients, who did not receive NAC showed only a slight insignificant increase. Comparable results were reported in a number of animal studies where administration of NAC was associated with a significant increase in GSH levels with preservation of antioxidant enzymes activity [20,21]. NAC might be one of the best antioxidants against OP poisoning, because it acts as a direct scavenging agent of oxygen-free radicals, prevents the induction of pro-inflammatory genes and is easily deacetylated to L-cysteine required for the production of GSH and thus can replenish intracellular glutathione stores [34].

Although the baseline levels of each of MDA and GSH were significantly different in the study groups, MDA levels were considerably reduced and GSH levels were notably elevated after NAC treatment. Both events signify the effectiveness of NAC as an antioxidant reducing the lipid peroxidation products and enhancing the endogenous antioxidant system. On the other hand, no such effects were seen in group B patients who did not receive NAC.

In the current study, patients on oral NAC therapy required less atropine doses, which coincides with an earlier study [14] using intravenous NAC administration. Several studies on OP exposure reported inhibition of both acetylcholinesterase and butyrylcholinesterase activities and its association with oxidative stress [8–10,14]. Hence, restoration of GSH stores besides direct scavenging of ROS may possibly explain the ameliorating effect of NAC on the inhibited cholinesterase. This could be responsible for reducing atropine requirements in NAC-treated patients [34]. Furthermore, inhibition of

acetylcholinesterase enzyme and the subsequent accumulation of the neurotransmitter acetylcholine and continued stimulation of muscarinic receptors may result in disordered calcium metabolism. Intracellular hypercalcaemia may facilitate formation of ROS that may inactivate the thiol-dependent calcium pump, which in turn aggravates the hypercalcaemia [35]. Therefore, by reducing oxidative stress, NAC may ameliorate the signalling pathways related to muscarinic receptors. This could be another mechanism explaining why NAC therapy has decreased the need for atropine. Atropine dosage regimen for acute OP poisoning in adults has never been studied in a randomized, controlled trial, and there is considerable variation in textbook recommendations. The most important end-point for adequate atropinization is clear lungs and reversal of the muscarinic toxic syndrome [36]. Reducing the dose of atropine is an important clinical issue as its administration in high doses is frequently associated with dangerous adverse health effects.

In the present study, no mortality occurred in both groups and all patients were cured after treatment. The absence of mortality in the current study may be related to differences in pattern and severity of poisoning depending on various factors such as the type of OP compound consumed, its amount, the time interval before hospitalization, the availability of effective treatment and the general health of the patient [37]. While Shadnia *et al.* [14] reported that NAC administration to OP-poisoned patients decreased the duration of hospitalization, the current study revealed no significant difference between the study groups regarding the duration of hospital stay.

Conclusion

N-acetylcysteine may safely be used as an adjuvant to conventional treatment of acute OP poisoning as no major adverse effects were reported with its use. Although it had no significant effect on hospitalization length, its use may provide an added benefit through reduction of atropine requirements and hence the proposed adverse effects resulting from larger atropine doses used in these cases of poisonings. Further studies in a larger number of patients are required before a conclusion can be made about the efficacy of NAC.

Limitations

The current study limitations include the small sample size; however, this was a pilot study assessing the safety and effectiveness of NAC in acute OP poisoning, and the results of

which should pave the way for larger trials recruiting patients based on sample size calculation. We consider that masking was lacking because healthcare providers may have noticed which patients received the NAC because of its characteristic smell. In addition, the study participants were randomized and their selection was based on certain eligibility criteria. Unfortunately, only one severe case was eligible for inclusion during the period of the study. The primary outcome, which was mortality, required severe OP poisoning cases, and as the study participants were mostly mild or moderate, there were no death events and we were unable to determine the effect of NAC on mortality. Furthermore, no measurement of acetylcholinesterase activity was carried out; however, determination of butyrylcholinesterase activity was particularly useful because of its sensitivity and wide availability.

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