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ORIGINAL ARTICLE

Evaluation of Oxidative Stress in Combination Therapy with D-penicillamine and N-Acetylcysteine (NAC) in Lead Poisoning in Opium Addicts

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Abstract

Background: N-acetylcysteine (NAC) is a putative antioxidant and has gained attention as a promising agent for chelating heavy metals including lead. Considering the animal studies results, we hypothesized that adding NAC to the treatment regimen may improve the success of treatment with lead chelators.
Method: A total of 46 patients who were lead-poisoned opioid addicts were divided into two groups randomly and treated with d-penicillamine (DP, 1g/day in four equal divided doses) and NAC+DP (1 g/day + 150 mg/kg/day). The efficacy of treatment was evaluated by hospitalization period. Meanwhile, the oxidative stress parameters including lipid peroxidation, protein carbonyl, total antioxidant capacity (TAC), glutathione concentration and super oxide dismutase (SOD) activity were determined on admission and discharge and compared with healthy normal controls.
Results: Hospitalization period was not different between the two groups. Treatment with DP and DP+NAC significantly decreased oxidative stress in patients. On the discharge day, the SOD activity and TAC were significantly higher in DP+NAC group in comparison with the DP group.
Conclusion: Although NAC recovers antioxidant capacity, the advantages of NAC in improvement of DP efficacy in lead poisoning is questionable. Further studies with larger sample size and combination with other chelators are recommended.

Keywords: D-penicillamine; Lead poisoning; N-acetyl cysteine; Opium addicts

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INTRODUCTION

Lead is a toxic heavy metal which has no essential function in human body. Lead poisoning is a major health problem throughout the world and it is estimated to account for 0.6% of the global burden of disease, with the highest burden in developing regions (1, 2). Even though the lead content of the Earth's crust is low, the anthropogenic activities such as mining, use of leaded petrol (gasoline), production of lead-acid batteries and paints, and plumbing materials and alloys are the main cause of widespread existence of lead in the environment (1).

Patterns and sources of exposure to lead are greatly different among the countries and even among the places within a country (3, 4). In this regard, consumption of adulterated opium has uniquely been one of the major sources of lead poisoning in Iran (5-9). To gain more benefits, opium smugglers add different adulterants including lead and thalium to opium to increase its weight (4, 10-14).

The most common symptoms of lead toxicity in opioid abusers are abdominal pain, anemia, weight loss, gastrointestinal complaints, fatigue, peripheral neuropathy, musculoskeletal complaints with muscle weakness, anorexia, and multi-organ dysfunction in kidney, liver, and CNS (3, 4). Non-specific clinical manifestations in lead poisoning, such as anemia, abdominal pain, constipation and encephalopathy can lead to hospitalization and prolonged hospital stay, thereby imposing considerable burden on patients and hospital.

The first step in the treatment of lead toxicity is ceasing exposure and oral or parenteral chelating therapy using dimercaprol, calcium disodium ethylene diamine tetra acetic acid (CaNa2EDTA), DP and succimer depending on the blood lead level in symptomatic patients (15). Beside these

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two principal approaches, other advices and medications have been recommended as follow: correcting dietary deficiencies, administration of thiamine, vitamin C, vitamin E and garlic oil for decreasing lead-induced oxidative damage and enhancing lead elimination from the body (15-17). In addition, there are some promising animal studies on rats which have shown the effectiveness of antioxidants such as methionine, alpha-lipoic acid, n-acetylcysteine and homocysteine for the treatment of lead toxicity (18-20).

N-acetylcysteine (NAC), derived from the amino acid cysteine, is a well-known antioxidant which has been used commonly in the treatment of respiratory diseases, psychiatric and neurologic disorders (21-25). NAC is a promising therapeutic antioxidant chelator agent for treatment of lead poisoning (26-28).

Considering the possible therapeutic effects of NAC in lead poisoning, the present study was conducted to evaluate therapeutic effects of NAC in combination with DP on lead poisoning in opium addicts. We further hypothesized that the protective effect of these drugs could be related to its antioxidant effects.

METHODS

Study design

A total of 46 opium dependent patients with signs and symptoms of lead poisoning admitted in Afzalipour Hospital, Kerman, Iran between April 2016 and July 2016 were enrolled in this study. Opium dependency was evaluated according to the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DMS-IV) criteria (29). Patients were included in the study if they were opium dependent, had lead poisoning clinical symptoms confirmed by laboratory tests and blood lead level (BLL) > 20 μg/dl (8, 29, 30), and over 18 years old. The local ethics committee approved the research protocol (Ethical code: 96-17), and patients were enrolled if informed consent was obtained. Patients were excluded if they met any of the following exclusion criteria: age <18 years, allergy to NAC or DP, occupational exposure to lead in workplace, concomitant poisonings, pregnant or lactating, BLL <20 μg/dl, severe organ dysfunction (n=1), a history of previous metabolic diseases known to generate oxidative stress, severe psychological complications during treatment (n=1), received any medication with antioxidant activity such as complementary antioxidants, vitamins, and selenium in the previous 48 h, inability to obtain verbal and written consent, and advanced malignancy (n=1). Clinical characteristics of patients like assessment of the level of consciousness and vital signs on admission were also registered. As a control for oxidative stress parameters, forty healthy non-smoker controls were recruited in this study. A questionnaire was used to obtain demographic data, information on occupation and medical history, job description, socio-economic status, and lifestyle of both groups. All patients received DP as a lead chelating agent. Twenty-eight patients underwent treatment with DP plus NAC as an antioxidant agent randomly. The doses of DP and NAC were 1 g/day in 4 equal divided doses and 150 mg/kg/day, respectively. In all patients, hematological tests including complete blood count (CBC), prothrombin time (PT) and partial thromboplastin time (PTT) tests, liver function tests including alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, and bilirubin tests, cholesterol test, and serum iron and total iron-binding capacity (TIBC) were measured on hospital admission and on the day of discharge.

Sample collection

Venous blood sample was collected from each subject in sterile heparinized and plain vacutainers and transported to the laboratory under cold conditions. The plasma and serum were separated by centrifugation at 3500 rpm for 15 min at 4 °C and stored at -80 °C until the analysis of oxidative stress parameters.

Determination of oxidative stress parameters

Lipid peroxidation (LPO) was estimated in serum as the reaction of lipid peroxides with thiobarbituric acid at low pH and high temperature. The resulting pink complex was measured at 532 nm (31). Superoxide dismutase (SOD) activity was measured according to the method of Marklund (32) with some modifications (33, 34) and expressed as Units (U). The serum concentration of GSH was measured spectrophotometrically at 412 nm using DTNB as the reagent (35) with minor modifications. Serum protein carbonyl (PrC) levels were measured on the basis of reaction between protein carbonyls with DNPH and formation of protein hydrazones with maximum absorbance at 365 nm (36). The serum total antioxidant capacity (TAC) was determined by measuring the ferric reducing ability of plasma in the presence of TPTZ (37) with some modifications (31).

Statistical analysis

All the data are presented as mean±SD or percent. Assessment of the normality of continuous data was relying on statistical tests (Kolmogorov-Smirnov test) and visual inspection (p-p plots and histogram). Student's *t* test was used for analysis of continuous variables, and χ^2 test was used for categorical variables. Non-normal distributed data were expressed as median and inter quartile range (IQR) and the differences between DP and DP+NAC group and these two groups with the control were respectively analyzed using Mann-Whitney U test and Kruskal–Wallis analysis of variance. P-value less than 0.05 was considered statistically significant.

RESULTS

The demographic data of the patients are listed in Table 1. Forty-three opioid abusers with symptoms of lead poisoning were involved in this study. All the cases had the clinical symptoms of lead poisoning confirmed by clinical toxicologists, and their BLL was above 20 μg/dl. There were no significant differences between BLL, age, gender, smoking habit, duration of opioid consumption, and grams of opioid consumption. These results show that the cases in both groups were selected randomly with no significant differences in their demographics. The duration of hospitalization was 7.1±3.1 and 7.6±3.3 days for DP and DP+NAC groups, respectively. The age of controls was 51.1±18.3 years old.

Table 1. Demographic data of patients

	DP (N=15)	DP+NAC (N=28)	Sig.*
Age (yrs.)	42.8±14.9	44.3±14.4	0.7
Sex, M/F (%)	11/4(73/27)	17/11(61/39)	0.41
Cigarette smoker, n (%)	6(40)	13(46)	0.67
BLL, median(IQR, range) (μg/dl)	55 (52,29-120)	55.3(63,21-182)	0.8
Hospitalization (day)	7.1±3.1	7.6±3.3	0.45
Duration of opioid use (yrs.)	5 (1-25)	8 (1-40)	0.5
Amount of opioid, median (IQR, range) (g/day)	2(3.5;0.5-12)	2.7(3.5;0.5-20)	0.8
Route of opioid use			
Snorting	0 (0%)	1 (4%)	0.5
Swallowing	11 (73%)	23 (82%)	
Both	4 (27%)	4 (14%)	

DP: d-penicillamine; NAC: n-acetyl cysteine; BLL: blood lead level
* P-value in Student's t-test, Mann-Whitney U test, and χ^2 test.

Table 2. Clinical symptoms on admission

Symptom	Total (N=43), n(%)	DP (n=15), n(%)	DP+NAC (n=28), n(%)	Sig.
Consciousness				
Awareness	27(63)	9(60)	18(64)	
Verbal	11(25)	5(33)	6(21)	0.49
Painful	2(5)	1(7)	1(4)	
Unconscious	3(7)	0(00	3(11)	
Weakness	25(58)	10(67)	15(54)	0.4
Lethargy	12(26)	3(20)	9(32)	0.4
Constipation	35(83)	13(87)	22(79)	0.5
Paresis	8(16)	1(7)	7(25)	0.1
Drowsiness	10(22)	2(14)	8(29)	0.2
Headache	7(17)	3(20)	4(14)	0.6
Insomnia	17(38)	5(33)	12(43)	0.5
Abdominal cramp	39(91)	14(93)	25(89)	0.6
Myalgia	5(12)	2(13)	3(11)	0.8
Colic	9(19)	2(13)	7(25)	0.4
Memory disorder	8(17)	2(13)	6(21)	0.5
Peripheral neuropathy	2(3.5)	0(0)	2(7)	0.3
Anorexia	35(83)	13(87)	22(79)	0.5

The clinical characteristics of cases on admission are listed in Table 2 and 3. The most common symptoms of the cases were abdominal cramps (91%), constipation (83%), anorexia (83%), weakness (58%), insomnia (38%), lethargy (26%), and colic (19%). The frequency of these symptoms was not significantly different in DP and DP+NAC cases. Table 3 shows that the laboratory parameters on admission were not different between the two groups.

Table 4 presents the data of oxidative stress parameters in DP and DP+NAC groups on admission and their comparison with the controls. A Kruskal-Wallis H test showed that there was a statistically significant difference in LPO between the different groups, $\chi^2(2) = 44.7$, $p = 0.000$, with a mean rank LPO of 23.7 for control, 61.9 for DP and 57.4 for DP+NAC. TAC was significantly different between the different groups, $\chi^2(2) = 60.7$, $p = 0.000$, with a mean rank TAC of 62.5 for control, 21.7 for DP and 21.4 for DP+NAC. SOD activity was significantly low in patients, $\chi^2(2) = 55.9$, $p = 0.000$, with a mean rank SOD of 62.5 for control, 23.4 for DP and 22.7 for DP+NAC. There was a significant increase in GSH concentration in the two groups, $\chi^2(2) = 7.3$, $p = 0.026$, with a mean rank GSH of 46.8 for DP and 49.8 for DP+NAC in comparison with the controls. PC was significantly high in the patients in both groups, $\chi^2(2) = 19.5$, $p = 0.000$, with a mean rank PC of 30.0 for control, 49.9 for DP and 54.9 for DP+NAC.

Table 5 presents the serum oxidative stress biomarkers in the patients treated with DP and DP+NAC. The lead-poisoned cases show significant oxidative stress (increase in lipid peroxidation, protein carbonyl and decrease in the levels of total antioxidant capacity, and SOD activity). Treatment of cases with DP and DP+NAC significantly decreased LPO and protein carbonyl markers of oxidative stress at discharge. However, TAC was decreased at discharge in both groups. There were no significant differences between oxidative stress parameters in DP and DP+NAC groups.

DISCUSSION

Several line of evidence suggests that n-acetyl cysteine could be effective in lead poisoning (18, 26, 38). So, in this study, the combined effect of NAC on the PD was investigated in lead-poisoned opium addicts. The results of this study indicate that even though NAC restores the antioxidant capacity, the clinical effectiveness of DP does not improve.

The results presented here have shown that lead exposure

Table 3. Clinical characteristics of patients on admission

	DP (N=15)	DP+NAC (N=28)	Sig.
Consciousness			
Aware, n (%)	9(60%)	18(64%)	0.5
Verbal, n (%)	5(33%)	6(21%)	
Painful, n (%)	1(7%)	1(4%)	
Unconscious, n (%)	0 (0%)	3(11%)	
SBP, mean±SD	119.6±31.1	125.4±21.3	0.72
DBP, mean±SD	71.6±10.1	77.46±8.9	0.45
Pulse rate, mean±SD	91.2±18.2	92.3±15.2	0.01
Temperature (◦C), mean±SD	35.8±3.9	37.2±0.6	0.15
RBC	4.2±0.8	4.1±1.0	0.74
MCV	26.6±2.7	24.2±3.2	0.2
MCH	26.6±2.7	24.2±3.2	0.017
HCT	34.5±9.7	34.5±9.7	0.68
Hb	10.5±4.1	9.7±2.8	0.51
BUN	37.3±16.1	36.4±16.6	0.86
Cr (mg/dl)	1.0±0.39	1.0±0.28	0.82
Bili total (mg/dl)	1.2±0.63	1.5±1.0	0.32
Bili direct (mg/dl)	0.3±0.18	0.3±0.19	0.35
PT	14.8±3.3	13.5±1.2	0.16
PTT	32.1±3.2	33.8±5.1	0.24
Iron	72(56)	83(73)	0.64
TIBC	345±74	326±62	0.37
AST (IU), median (IQR)	40(61)	54(48)	0.68
ALT (IU), median (IQR)	30(40)	33(39)	0.44
ALP (IU), mean±SD	208±106	260±146	0.23
Amylase	67(40)	71(50)	0.97

Comparisons were made by using Student's-sample t-test. Data are expressed as Mean±SD
MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

induces oxidative stress which was indicated by increased lipid peroxidation and protein carbonyl levels and decreased SOD activity and TAC. However, the only exception is GSH level which was higher in the exposed patients. Accumulating evidence over the past years has identified many of the important pathogenic mechanisms of lead, yet the precise molecular mechanisms involved are not fully understood (39). The main reported mechanism for lead toxicity is induction of reactive oxygen species (ROSs) production and oxidative stress. Increase in ROS starts a chain reaction and may result in lipid peroxidation, oxidation of DNA, RNA and proteins, and thereby significant damage to cell structures, cumulatively known as oxidative stress (39, 40. Lead exposure can induce oxidative stress through different mechanisms. Lead can inhibit the δ-aminolevulinic acid dehydrase (ALAD) enzyme and cause accumulation of δ-aminolevulinic acid (ALA). Auto-oxidation of accumulated ALA induces generation of ROS (41-43). Lead exposure can also create oxidative stress by inhibition of antioxidant enzymes and also depletion of glutathione reserves in the cells (27, 39). Regarding this mechanism of lead, administration of antioxidants for restoring the cell's antioxidant capacity has been considered as a plausible remedy (28, 44). In several animal studies, there are promising reports regarding combination therapy with a chelator and antioxidants in lead poisoning (45-47). In these studies, mostly conducted by Flora et al., the advantages of adding an antioxidant such as NAC (45), alpha-lipoic acid (47), melatonin (45), and gossypin (48) to a lead chelator have been shown (20).

Our findings, resulting from the only study conducted on human according to the search of literature, show that combined therapy with NAC does not improve the efficacy of DP and oxidative stress in lead poisoned addicts. This shows that decrease in the lead concentration might be the main mechanism for reduction in oxidative stress in lead poisoning. While NAC has been found as metal and specially lead chelator in several in vitro studies (26, 27), other in vivo studies in animals have failed to find its effect on excretion of lead and its potential use for lead chelation therapy (49-51). The antioxidant actions of NAC have been attributed to its ability to stimulate glutathione (GSH) synthesis through

Table 4. Oxidative stress parameters in controls and lead-poisoned opioid addicts treated with DP and DP+NAC on admission

	Healthy controls (N=40)	On admission		*P values
		DP (N=15)	DP+NAC (N=28)	
LPO (μM)	0.41(0.17)	0.94(0.4)	0.80(0.34)	0.000
PC (mM)	0.26(0.15)	0.33(0.05)	0.36(0.07)	0.000
TAC (mM)	1.11(0.21)	0.24(0.32)	0.18(0.23)	0.000
GSH (μM)	14.9(5.2)	16.3(2.6)	17.3(4.8)	0.026
SOD (U)	30.52(4.8)	4.8(2.9)	7.3(1.72)	0.000

Data are expressed as median (Inter Quartile Range; IQR).
*P value of DP and DP+NAC groups at admission vs. the control group. Data are analyzed using Kruskal-Wallis H test. DP: d-penicillamine; NAC: n-acetyl cysteine; LPO: lipid peroxidation; PC: Protein carbonyl; TAC: total antioxidant capacity; GSH: glutathione; SOD: superoxide dismutase.

Table 5. Oxidative stress parameters at discharge in lead-poisoned opioid addicts treated with DP and DP+NAC

	On discharge		*P-values
	DP (N=15)	DP+NAC (N=28)	
LPO (μM)	0.49(0.3)	0.52(0.23)	0.65
PC (mM)	0.22(0.03)	0.29(0.09)	0.000
TAC (mM)	0.07(0.11)	0.15(0.13)	0.042
GSH (μM)	19.4(3.9)	18.2(4.2)	0. 957
SOD (U)	6.8(1.1)	7.9(1.2)	0.007

Data are expressed as median (Inter Quartile Range; IQR).
* Data are analyzed using Mann-Whitney U test.
DP: d-penicillamine; NAC: n-acetyl cysteine; LPO: lipid peroxidation; PC: Protein carbonyl; TAC: total antioxidant capacity; GSH: glutathione; SOD: superoxide dismutase.

providing cysteine for GSH synthesis and thereby maintain and replenish GSH levels in the cells, direct scavenging of free radicals, and efficient reduction of disulfide bands in proteins (25, 52, 53). A contradictory finding in the study is that the level of carbonyl protein was higher in patients treated with NAC+DP. There are reports that show high concentrations of NAC act as a pro-oxidant (25) which can initiate an enhanced generation of both reduced glutathione and oxidized glutathione and enhanced production of reactive oxygen species, along with carbonylation and glutathionylation of the cellular proteins (54). Inhibition of SOD enzymes has been reported in several experiments (55).

CONCLUSION

In conclusion, this study indicates that addition of NAC antioxidant to the protocol of chelation therapy with DP cannot improve the treatment efficacy in lead poisoning. Further studies with larger sample size and combination of NAC with other chelators of lead could give more conclusive results.

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