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ORIGINAL ARTICLE

Effect of treatment with N-acetylcysteine on non-enzymatic antioxidant reserves and lipid peroxidation in workers exposed to lead

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Abstract

There are no published studies examining the effects of N-acetylcysteine (NAC) administration on the non-enzymatic defence systems in humans exposed to lead. In view of this, it was decided to measure the levels of uric acid (UA), albumin, bilirubin and alpha-tocopherol before and after treatment with NAC. An estimation was also made of the degree of oxidative stress by measuring the ferric reducing ability of plasma (FRAP), the levels of conjugated dienes (CD) and lipid hydroperoxides (LHP). Male employees who worked with lead were randomized into two groups. The first group included workers who were not administered any drugs (n=49), while the second group (n=122) consisted of workers who were treated with NAC at three different doses (200 mg, 400 mg and 800 mg) for 12 weeks. The administration of NAC (400 mg, 800 mg) resulted in significant decreases in the LHP levels. Similarly, a strong tendency toward lower levels of CD was observed in the same groups. The UA levels were significantly lower only in the group receiving the 200 mg dose of NAC. However, the alpha-tocopherol levels were significantly elevated after treatment with NAC (400 mg, 800 mg). NAC administration did not significantly affect the levels of bilirubin and albumin, but a tendency toward higher values was observed for FRAP. NAC reduced the extent of lipid peroxidation in a dose-dependent manner. Elevated concentrations of alpha-tocopherol may have enhanced the beneficial effects of NAC. Treatment with NAC may contribute to the restoration of non-enzymatic antioxidant reserves when administered to lead-exposed workers.

Keywords

lead exposure, N-acetylcysteine, antioxidants, human

INTRODUCTION

N-acetylcysteine (NAC) is a low molecular weight compound administered to neutralize the deleterious effects of free radicals. NAC raises the intracellular concentration of cysteine and hence of reduced glutathione (GSH), which acts as an important endogenous antioxidant. Moreover, NAC has direct scavenging properties *in vitro* against hydroxyl radicals and hypochlorous acid [1]. NAC is rapidly absorbed following oral dosing and reaches its peak concentrations in the blood within 1 hour. After passing through the intestine and liver, NAC is transformed into a variety of metabolites. Consequently, NAC has been shown to have multiple therapeutic benefits [2].

NAC has been used in clinical practice since the 1960s when it was introduced as a mucolytic agent for the treatment of respiratory diseases, such as chronic bronchitis and cystic fibrosis. Since the late 1970s, NAC has been administered as an antidote for the therapy of acute acetaminophen intoxication [3]. Currently, NAC is being studied for use in many disorders, such as chronic obstructive pulmonary disease (COPD), contrast-induced nephropathy, influenza, idiopathic pulmonary fibrosis, polycystic ovary syndrome [4] and lead poisoning [5].

Lead induces a broad range of physiological and biochemical dysfunctions [5]. Exposure to this metal affects many body systems and leads to hematopoietic, cardiovascular, immunological, renal and hepatic dysfunction. The kidney is particularly susceptible to lead toxicity, resulting in nephropathy, proximal tubular damage, glomerular sclerosis, and lowered glomerular filtration rate. In addition, the effects of lead toxicity on the cardiovascular system include hypertension, coronary heart disease, stroke and peripheral arterial disease [6,7].

Recent studies have demonstrated the potential of lead to induce oxidative stress. The evidence also indicates that lead acts through multiple mechanisms. First, lead has a high affinity for thiol groups, and it forms thiolates with the thiol groups of cysteine. Lead also forms less stable complexes with other amino acid side chains. Lead exposure can also result in the depletion of glutathione and the inhibition of several enzymes having functional thiol groups, such as delta-aminolevulinic acid dehydratase [8]. Second, lead generates reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radical, singlet oxygen and hydroxyl radical [9]. ROS attack all cellular macromolecules. The polyunsaturated fatty acid residues of phospholipids are extremely sensitive to oxidation and undergo a process of

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peroxidation [7]. This results in the formation of conjugated dienes, lipid hydroperoxides and degradation products, such as malondialdehyde (MDA) [10].

Studies on lead-exposed rats have shown that the administration of NAC decreased the levels of MDA and oxidized glutathione (GSSG); it was also effective in restoring reduced glutathione (GSH) in the blood [11]. There are apparently no published studies on the effects of NAC administration in humans exposed to lead. Taking into account that NAC has a high toxicity threshold and a long history of safe clinical use, the decision was made to evaluate the effects of NAC administration in lead-exposed workers.

The accumulated data about the interactions between lead and the non-enzymatic defence systems are fragmentary and inconsistent. In view of this, it was decided to simultaneously measure the levels of uric acid, albumin, bilirubin and alpha-tocopherol before and after NAC treatment. The degree of oxidative stress and lipid peroxidation, were also estimated by determining the ferric reducing ability of plasma, the levels of conjugated dienes and the quantities of lipid hydroperoxides.

MATERIALS AND METHOD

Study population. The examined population included male employees in the southern region of Poland who worked with lead. Their mean age was 42.5 ± 8.66 years and had been exposed to lead for an average of 18.1 ± 9.46 years. To determine the degree of lead exposure, the blood concentrations of lead (PbB) and zinc-protoporphyrin (ZPP) were measured. These measurements were taken, on average, every 3 months during the 2 years of observation. The mean blood concentrations of lead (PbB_{mean}) and zinc-protoporphyrin (ZPP_{mean}) were calculated from the collected data.

Inclusion criteria were defined as follows: occupational exposure to lead (PbB_{mean} > 20 μg/dl and ZPP_{mean} > 2.5 μg/g Hb), a negative physical examination (no symptoms and signs of any disease, such as elevated blood pressure, abnormal pulse, auscultatory signs, elevated body temperature etc.), and no past or present history of any of chronic disease. Exclusion criteria were exhibition of any contraindications to the treatment with NAC.

The examined population (n=171) was randomized into 2 groups. The first group included workers who were not administered any antioxidants, drugs, vitamins or dietary supplements (reference group, n=49). The second group consisted of 3 subgroups of workers who were treated orally with NAC (Fluimucil®, Medagro) at 3 different doses for 12 weeks (n=122). The subdivision of the second group was according to the NAC dose. The first subgroup included 40 workers who were administered 200 mg of NAC once a day (NAC 200 subgroup), the second subgroup included 44 workers who were administered 200 mg of NAC twice a day (NAC 400 subgroup), and the third subgroup included 38 workers who were administered 400 mg of NAC twice a day (NAC 800 subgroup).

Blood from all the examined workers was collected at the beginning of the study and after 12 weeks of treatment or observation. The 2 blood samples from each participant were examined for the levels of uric acid (UA), albumin, bilirubin in serum and alpha-tocopherol in the plasma. The ferric reducing ability of the plasma (FRAP), the levels of conjugated dienes (CD) in plasma and the levels of lipid hydroperoxides (LHP) in the serum were also determined.

The experimental protocol was reviewed and approved by the Bioethics Committee of the Medical University of Silesia in Katowice (No. NN-6501–36/I/06).

Laboratory procedures. Whole blood was used for the analysis of the lead (PbB) and zinc-protoporphyrin (ZPP) levels.

Graphite furnace atomic absorption spectrophotometry (Unicam 929 and 939OZ Atomic Absorption Spectrometers with GF90 and GF90Z Graphite Furnaces) was used to measure the PbB levels. Data were displayed as μg/dl.

The ZPP concentration was measured by means of the Aviv Biomedical haematofluorometer (Model 206). In this method, light is filtered by means of an interference filter transmitting at a wavelength of 415 nm. The excitation light is focused on a drop of blood. The emitted light passes through a narrow band interference filter transmitting at a wavelength of 596 nm. The instrument measures the ratio of ZPP as a fluorescent substance to the absorption of the light in the sample (haemoglobin). The results were expressed as μg ZPP per gram of haemoglobin (μg/g Hb).

The concentration of CD was measured according to the method by Corongiu et al. [12] using a spectrophotometer (Shimadzu, Model A160). The method by Södergren et al. [13] was used to measure the concentration of LHP. The results were expressed as μmol/l.

FRAP levels were determined by the Benzie and Strain method [14] using the biochemical analyzer EM 280 (Emapol, Poland). The data were shown as μmol/l. The level of alpha-tocopherol was measured by the Shearer method [15], incorporating high-performance liquid chromatography with a Spherimage 80 ODS2 column and a UV/Vis detector (Knauer, Germany). EuroChrom 2000 software (Knauer, Germany) was used to perform calculations. The results were shown as μmol/l. The concentrations of uric acid (UA), albumin and bilirubin were measured using the A25 biochemical analyzer (BioSystems, Spain), according to the manufacturer's instructions. The results for UA and bilirubin were expressed as μmol/l, while those for albumin expressed as g/l.

Statistical analysis. All statistical analyses were performed using Statistica 9.1 PL software. The statistical methods included the mean and standard deviation. The Shapiro-Wilk's test was used to verify normality, whereas Levene's test was used to verify the homogeneity of the variances. Statistical comparisons between groups used Student's t-test, a t-test with separate variance estimates or the Mann-Whitney U test. Dependent variables were analyzed using Student's t-test and the Wilcoxon rank-sum test. An alpha value of $p<0.05$ was considered statistically significant.

RESULTS

No significant differences in age, years of work, body mass index (BMI) or smoking habits were observed between the examined groups and the subgroups. There were also no differences in the PbB_{mean} and the ZPP_{mean} levels calculated before the treatment (Tab. 1).

The administration of NAC resulted in significant decreases in the LHP levels in the NAC 400 and NAC 800 subgroups

Table 1. Epidemiologic parameters, the mean blood lead level (PbB_{mean}) and the mean level of zinc protoporphyrin in blood (ZPP_{mean}) in a Pb-exposed population before treatment with N-acetylcysteine (NAC) and observation in the reference group. p value – in comparison to reference group

	Reference group n=49		NAC-total n=122			NAC-200 n=40			NAC-400 n=44			NAC-800 n=38		
	mean	SD	mean	SD	p value	mean	SD	p value	mean	SD	p value	mean	SD	p value
age	40.9	8.25	42.5	8.66	0.288	40.6	8.49	0.848	43.1	8.81	0.218	43.7	8.55	0.131
years of work	15.2	8.57	18.1	9.46	0.067	17.1	9.71	0.325	18.4	9.50	0.088	18.7	9.33	0.074
BMI (kg/m^2)	27.1	3.21	27.0	3.61	0.902	27.3	3.63	0.766	26.4	3.56	0.364	27.34	3.66	0.707
smoking habits	55%		52%		0.756	55%		0.992	52%		0.787	50%		0.641
PbB_{mean} (µg/dl)	42.9	6.3	43.8	7.4	0.449	41.7	7.41	0.400	44.9	7.61	0.159	44.73	6.77	0.195
ZPP_{mean} (µg/g Hb)	7.96	4.00	8.04	3.33	0.896	7.63	3.54	0.686	8.34	3.20	0.616	8.11	3.29	0.847

Table 2. Oxidative stress intensity measured as the level of conjugated dienes (CD) in plasma and lipid hydroperoxides (LHP) in serum in a Pb-exposed population before and after 3 months of treatment with N-acetylcysteine (NAC) and observation in the reference group. p value – in comparison to reference group, p value* comparison between after and before treatment

	Reference group n=49		NAC-total n=122			NAC-200 n=40			NAC-400 n=44			NAC-800 n=38		
	mean	SD	mean	SD	p value	mean	SD	p value	mean	SD	p value	mean	SD	p value
CD concentration (µmol/l) before	145	24.1	154	29.6	0.076	151	21.2	0.260	157	33.1	0.192	153	33.2	0.203
CD concentration (µmol/l) after	152	27.4	145	25.5	0.096	148	22.2	0.485	145	28.4	0.239	140	25.2	**0.043**
p-value*	0.168		**0.014**			0.176			**0.064**			0.270		
Δ CD-p concentration	7.10	37.6	-8.76	37.7	**0.008**	-2.20	16.5	**0.049**	-11.5	44.8	**0.026**	-12.5	44.4	**0.029**
LPH concentration (µmol/l) before	20.1	7.26	21.1	11.1	0.657	19.3	7.26	0.718	21.7	11.49	0.529	21.6	13.0	0.596
LPH concentration (µmol/l) after	21.4	11.8	15.3	7.60	**0.002**	19.9	7.64	0.612	16.1	7.22	**0.025**	10.8	5.55	**<0.001**
p-value*	0.736		**<0.001**			0.330			**<0.001**			**<0.001**		
Δ LPH -s	1.38	8.45	-5.79	9.51	**0.001**	0.64	6.56	0.742	-5.55	7.38	**0.001**	-10.9	11.2	**<0.001**

compared to the reference group. Similarly, a strong tendency towards lower values of the CD levels was observed in the same subgroups (Tab. 2).

UA levels after NAC administration were significantly lower only in the NAC 200 subgroup compared to the reference group. However, the concentrations of alpha-tocopherol were significantly elevated after the treatment with NAC in the NAC 400 and NAC 800 subgroups compared to the levels at the beginning of the study. Only in the NAC 800 subgroup was the observed increase significant when compared to the reference group (Tab. 3).

Treatment with NAC did not significantly affect the bilirubin or albumin levels, while there was a tendency toward higher FRAP values (Tab. 3).

DISCUSSION

Thee presented study demonstrates that administration of higher doses of NAC (400 and 800 mg) significantly decreased serum levels of lipid hydroperoxides in workers chronically exposed to lead, while the uric acid was decreased only after dose of 200 mg of NAC. Additionally we showed elevated alpha-tocopherol plasma levels following NAC administration.

Both LHP and CD levels have been reported to increase due to the action of lead ions. Increased LHP concentrations were observed in the liver and serum of rats treated with lead acetate [16,17]. However, Adonaylo and Oteiza [18] observed elevated CD levels when investigating the effect of lead on lipid oxidation in liposomes. In the presented study, it is shown that after NAC administration the levels of LHP did not change in the NAC 200 subgroup, but decreased significantly by 26% and 50% in the NAC 400 and NAC 800 subgroups, respectively. These results suggest that NAC reduced lipid peroxidation in a dose-dependent manner. The effect of treatment with NAC on the CD concentrations was less significant. However, a tendency toward lower CD levels was observed. *In vivo* and *in vitro* studies in animals are in accordance with the presented study because many authors have observed that NAC administration significantly decreased MDA levels, which served as a lipid peroxidation biomarker [11]. The potency of NAC to reduce lipid peroxidation intensity in lead exposure may be due to its ability to scavenge free radicals and stimulate synthesis of glutathione which serves as a major thiol antioxidant of the human body [1]. Besides, it is possible that NAC reduces the interactions of lead with thiol groups of antioxidant enzymes or even chelates lead ions, although the chelating properties of NAC are controversial.

Alpha-tocopherol possesses the highest biological activity in the group of 8 naturally-occurring compounds collectively referred to as vitamin E. Alpha-tocopherol limits the propagation of lipid peroxidation and is required to protect biological membranes and lipoproteins from oxidative stress [19]. Rendón-Ramirez et al. [20] reported

Table 3. Non-enzymatic antioxidant concentrations, uric acid (UA), albumin, bilirubin in serum, alpha-tocopherol in plasma and ferric reducing ability of plasma (FRAP), in a Pb-exposed population before and after 3 months of treatment with N-acetylcysteine (NAC) and observation in the reference group. p value – in comparison to reference group, p value* comparison between after and before treatment

	Reference group n=49		NAC-total n=122			NAC-200 n=40			NAC-400 n=44			NAC-800 n=38		
	mean	SD	mean	SD	p value	mean	SD	p value	mean	SD	p value	mean	SD	p value
FRAP (µmol/l) before	514	72.2	524	119	0.646	492	75.0	0.220	535	148	0.442	536	105	0.294
FRAP (µmol/l) after	517	72.5	558	116	**0.040**	534	73.8	0.368	553	94.5	0.060	585	160	**0.022**
p-value*	0.653		**0.018**			**0.026**			0.269			0.322		
Δ FRAP-R	3.15	92.6	34.5	156	0.245	41.9	83.6	0.081	18.7	157	0.593	48.3	198	0.210
UA concentration (µmol/l) before	324	85.3	342	100	0.285	339	57.0	0.373	345	118	0.329	342	116	0.426
UA concentration (µmol/l) after	330	79.3	314	68.6	0.204	301	61.6	0.067	330	71.8	0.990	310	70.1	0.222
p-value*	0.464		**<0.001**			**<0.001**			0.381			0.189		
Δ UA	5.24	92.6	-27.7	89.6	**0.016**	-37.3	64.3	**0.016**	-15.3	86.4	0.273	-32.0	114	0.096
α-tocopherol conc. (µmol/l) before	18.9	6.77	20.8	8.80	0.189	17.8	6.64	0.456	21.5	9.57	0.154	23.0	9.14	**0.024**
α-tocopherol conc. (µmol/l) after	19.7	8.95	25.2	9.74	**0.001**	20.4	9.34	0.732	24.9	7.70	**0.006**	29.9	10.1	**<0.001**
p-value*	0.759		**<0.001**			0.246			**0.029**			**<0.001**		
Δ α-tocopferol	0.79	9.50	4.33	10.1	**0.040**	2.66	9.08	0.384	3.41	9.96	0.222	6.89	11.0	**0.009**
Bilirubin concentration (µmol/l) before	14.7	6.09	16.0	10.9	0.472	14.9	9.11	0.919	16.5	11.8	0.379	16.5	11.8	0.377
Bilirubin concentration (µmol/l) after	16.1	8.31	16.1	8.36	0.957	13.8	8.39	0.079	17.4	7.87	0.463	16.8	8.61	0.724
p-value*	0.321		0.344			0.514			0.141			0.433		
Δ bilirubin	1.46	9.05	0.10	11.8	0.495	-1.02	9.66	0.235	0.94	12.3	0.826	0.29	13.2	0.643
Albumin concentration (g/l) before	46.1	3.26	43.7	3.92	**<0.001**	45.7	3.07	0.566	42.8	3.56	**<0.001**	42.6	4.42	**<0.001**
Albumin concentration (g/l) after	44.7	3.87	43.6	4.24	0.121	45.9	3.59	0.163	42.9	3.79	**0.029**	42.0	4.49	**0.005**
p-value*	0.031		0.433			0.959			0.955			0.180		
Δ Albumin	-1.33	4.15	-0.04	3.61	**0.048**	0.22	2.83	**0.041**	0.16	4.38	0.095	-0.58	3.33	0.383

that vitamin E administration to rat erythrocytes treated with lead decreased the oxidative damage to lipids and prevented the inhibition of delta-aminolevulinic dehydratase activity. Consistently, Patra et al. [21] observed decreased MDA levels in the livers and brains of rats administered lead and alpha-tocopherol compared to rats administered only lead. However, a study by Ergurhan-Ilhan et al. [22] showed that even low exposure to lead (PbB = 7.9 ± 5.2 µg/dl) may result in decreased levels of serum alpha-tocopherol in humans. In contrast, Prokopowicz et al. [23] indicated that occupational lead-exposure is negatively correlated with the concentration of gamma-tocopherol rather than with alpha-tocopherol. However, Caylak et al. [24] observed decreased levels of vitamin E in rats orally exposed to lead acetate. In this report, NAC administration did not restore the vitamin E levels. In contrast, the results of the presented study indicate that treatment with NAC increases alpha-tocopherol concentrations in lead-exposed workers in a dose-dependent manner. Concentrations of alpha-tocopherol in the NAC 400 and NAC 800 subgroups increased by 16% and 30%, respectively. These elevations of alpha-tocopherol may contribute to the simultaneously observed decreased levels of LHP.

Bilirubin is an end product in heme metabolism and is known to have toxic effects at high concentrations. In humans, under physiological conditions, bilirubin serves as a strong antioxidant, which acts synergically with alpha-tocopherol to protect lipids from oxidative damage [25]. Noriega et al. [26] showed that bilirubin administration decreases lipid peroxidation and increases glutathione levels and the activity of antioxidant enzymes in rats. Conversely, elevated plasma bilirubin levels in rats treated with lead acetate have been reported in several studies [27]. The current findings indicate that NAC administration did not significantly influence bilirubin levels in lead-exposed workers. Because bilirubin acts as an antioxidant, the normalization of its levels after NAC administration would not be desirable.

Nephropathy occurs most often at high levels of lead exposure (>60 µg/dl). In contrast, damage to the kidney at lower lead levels has been reported (~10 µg/dl). There are 2 types of renal functional abnormalities resulting from lead-exposure, acute nephropathy and chronic nephropathy. In acute nephropathy, a tubular transport mechanism is impaired and abnormal excretion of glucose, phosphates and amino acids are observed (Fanconi-like syndrome). In contrast, chronic nephropathy is much more severe

and is characterized by glomerular and tubulo-interstitial changes, which may cause renal breakdown, hypertension and hyperuricaemia [28].

A significant increase in the UA levels in lead-exposed workers with PbB = 29.1 μg/dl and PbB = 80.9 μg/dl was reported in studies by Khan et al. [7] and by Bener et al. [29], respectively. Positive correlations between blood lead levels and UA concentrations were also observed in humans by Hernández-Serrato et al. [30] and by Ehrlich et al. [31]. However, no association between blood lead and UA levels has been reported. Alternatively, Berrahal et al. [32] observed decreased UA levels in rats that were orally exposed to lead acetate. The authors of this study suggested that the hypouricaemia might occur because of decreased xanthine oxidase (XO) activity or due to lead-induced Fanconi-like renal tubular defects.

The decreased levels of UA, which were observed in the present study in the NAC 200 subgroup, and the tendencies toward lower UA levels in the remaining subgroups, suggest that NAC may exert protective effects on kidney function. Tayman et al. [33] reported decreased XO activity when investigating the effect of NAC on the development of necrotizing enterocolitis (NEC) in an experimental rat model. Therefore, it is possible that NAC could also inhibit XO activity in lead-exposed workers. In light of this, it is difficult to explain why 400 and 800 mg of NAC did not significantly affect the UA levels. Larger populations should be examined to verify the presented results. On the other hand, given that UA accounts for up to 60% antioxidative capacity of human blood [34], the limited influence of NAC administration on UA levels in the NAC 400 and NAC 800 subgroups could be beneficial.

Albumin is synthesized in the liver and constitutes approximately 60% of the total protein in plasma. The cysteine thiol groups of albumin determine the redox status of plasma and act as ROS scavengers. Nonetheless, albumin sequesters redox-active metals and has been proposed to have a thioredoxin-dependent lipid hydroperoxide reductase activity *in vitro* [34, 35]. Koo et al. [36] observed decreased albumin mRNA levels in rat liver after the administration of lead nitrate. These results are supported by the studies of Mikhail et al. [37] and Khan et al. [7], who observed decreases in serum albumin and total protein levels in lead tank welders (PbB = 42.19 μg/dl) and lead-exposed industrial workers. However, Al-Neamy et al. [38] did not observe any significant changes in the levels of albumin and total protein in industrial workers exposed to lead (PbB = 77.5 μg/dl). Accumulated data indicate that the observed reductions in albumin and protein levels may be caused by the lead-induced inhibition of protein biosynthesis. The results of the presented study suggest that NAC administration would not be able to restore albumin to the levels preceding lead depletion.

The antioxidant and reducing potentials of biological fluids may be characterized by the FRAP values [14]. Although NAC administration was effective in decreasing the markers of lipid peroxidation, FRAP levels remained unchanged in the examined subgroups. A tendency toward higher levels compared to the reference group was only observed in the NAC 200 subgroup. The lack of significantly changed FRAP values may be due to the quick utilization of the thiol substrates provided by NAC, which maintained a reduced level of lipid peroxidation.

CONCLUSIONS

When administered to workers chronically exposed to lead, NAC reduced lipid peroxidation in a dose-dependent manner. The simultaneously elevated concentrations of alpha-tocopherol may enhance the beneficial effects of NAC. However, the influence of NAC on the levels of UA, bilirubin, albumin and FRAP seems to be limited. In conclusion, NAC, when administered to lead-exposed workers, may contribute to the restoration of non-enzymatic antioxidant reserves.

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