Clinical Toxicology (2013), **51**, 480–486
Copyright © 2013 Informa Healthcare USA, Inc.
ISSN: 1556-3650 print / 1556-9519 online
DOI: 10.3109/15563650.2013.802797

informa
healthcare

CRITICAL CARE

The administration of N-acetylcysteine reduces oxidative stress and regulates glutathione metabolism in the blood cells of workers exposed to lead

SŁAWOMIR KASPERCZYK, MICHAŁ DOBRAKOWSKI, ALEKSANDRA KASPERCZYK, ALINA OSTAŁOWSKA, and EWA BIRKNER

Department of Biochemistry, Medical University of Silesia, Zabrze, Poland

Clinical Toxicology Downloaded from informahealthcare.com by Gazi Univ. on 08/19/14
For personal use only.

Context and objective. The aim of the study was to investigate whether treatment with N-acetylcysteine (NAC) is able to restore erythrocyte glutathione (GSH) content in workers exposed to lead. Additionally, we measured the leukocyte and erythrocyte activities of GSH-related enzymes, such as glutathione reductase (GR), glutathione-S-transferase (GST), and glucose-6-phosphate dehydrogenase (G6PD), and estimated the influence of NAC administration on oxidative stress intensity, which was measured as the lipofuscin (LPS) level in erythrocytes. *Methods.* The exposed population consisted of 171 healthy males randomly divided into four groups. Workers in the first group ($n = 49$) were not administered any antioxidants, drugs, vitamins, or dietary supplements, while workers in the remaining groups were treated with NAC at three doses for 12 weeks (1×200 mg per day, 2×200 mg per day, and 2×400 mg per day). All workers continued to work during the study. The blood of all examined workers was drawn two times: at the beginning of the study and after 12 weeks of treatment. *Results and conclusion.* Blood lead levels decreased significantly in all groups receiving NAC compared to those in baseline. Erythrocyte GSH concentrations were significantly elevated in workers receiving 400 and 800 mg of NAC compared to those in baseline by 5% and 6%, respectively. Erythrocyte G6PD activity was significantly elevated in workers receiving 200, 400, and 800 mg of NAC compared to those in baseline by 24%, 14%, and 14%, respectively. By contrast, there were no significant differences in leukocyte G6PD or leukocyte and erythrocyte glutathione reductase (GR) activities before and after treatment. Leukocyte GST activities decreased significantly after treatment in workers receiving 200 mg of NAC by 34%, while LPS levels decreased significantly in workers receiving 200, 400, and 800 mg of NAC compared to those in baseline by 5%, 15%, and 13%, respectively. In conclusion, NAC decreases oxidative stress in workers exposed to lead via stimulating GSH synthesis.

Keywords Lead poisoning; N-acetylcysteine; Oxidative stress; Glutathione; Glucose-6-phosphate dehydrogenase

Introduction

The widespread usage of lead in different industries is due to its unique properties, such as softness, high malleability, ductility, low melting point and resistance to corrosion. Lead interferes with a number of body functions and produces serious disorders, such as encephalopathy, anemia, nephropathy and hypertension. Lead not only generates reactive oxygen species (ROS), such as hydroperoxides, singlet oxygen, and hydrogen peroxide, but also depletes the antioxidant reserves.[1,2] Lead has a strong affinity for thiol groups. Because these groups are essential for the catalytic activities of many enzymes, lead is able to disrupt the function of enzymatic pathways, such as the heme biosynthesis pathway, and interfere with antioxidant enzymes.[1,3,4]

Lead-induced free radicals generation results in lipid peroxidation, disruption of cell membrane, protein oxidation, and oxidation of DNA and RNA. Therefore, it is suggested that administration of various antioxidants can prevent or diminish various toxic effects of lead.[1] Because there is no safe level of exposure to lead and complete control and prevention over lead-exposure is still far from being achieved, it is worth exploring whether antioxidants have the actual potential to restore the impaired pro-oxidant/antioxidant balance in lead poisoning.

Lead inactivates glutathione (GSH),[1] which is a major thiol antioxidant of the human body. This inactivation results in the synthesis of GSH from cysteine via the gamma-glutamyl cycle. However, this cycle is usually not effective in restoring the GSH supply.[1,3] Because N-acetylcysteine (NAC) provides a rate-limiting cysteine needed for GSH synthesis and reduces the interactions of lead with thiol groups,[5,6] it is theoretically a good candidate for the treatment of lead intoxication.

NAC is a thiol antioxidant that is rapidly absorbed following an oral dose. The plasma half-life of NAC is estimated to

Received 17 March 2013; accepted 1 May 2013.

Address correspondence to Sławomir Kasperczyk, M.D., Ph.D., Department of Biochemistry, Medical University of Silesia, ul. Jordana 19, 41-808 Zabrze, Poland. Tel/Fax. + 48 32 2722318. E-mail: kaslav@mp.pl

Clinical Toxicology Downloaded from informahealthcare.com by Gazi Univ. on 08/19/14
For personal use only.

be approximately 2.5 h.[7] NAC undergoes quick deacylation to cysteine, which is a rate-limiting precursor in the synthesis of intracellular reduced GSH.[5] The effectiveness of NAC as an antioxidant is attributed to its ability to reduce extracellular cystine to cysteine.[8] Apart from being a source of thiol groups, NAC can also act as a direct scavenger of reactive oxygen species; however, the reactivity of NAC with superoxide anion and hydrogen peroxide is being discussed.[7,9,10] The chelating properties of NAC are also controversial.[5,11]

Studies in vitro and in vivo on lead-exposed rats and mice showed that NAC administration normalizes the GSH level, decreases the level of the oxidized form of glutathione (GSSG), and reduces oxidative stress biomarker levels.[5,6,12–16] Therefore, it is worth exploring whether NAC administration would reverse the negative effects of lead-induced GSH depletion in humans because there is no study in the available literature on this topic.

In light of this, in the present study, we investigated whether treatment with NAC is able to restore erythrocyte GSH content in workers occupationally exposed to lead. We decided to also measure activities of GSH-related enzymes and estimate the influence of NAC administration on oxidative stress intensity.

Methods

Experimental design

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

The exposed population consisted of healthy male employees of zinc and lead works located in Miasteczko Śląskie, Poland. All of them were volunteers who were recruited by a specialist of occupational medicine during prophylactic medical examinations and provided informed consent to the study. The mean concentration of lead in the air at participants' workplaces was 0.083 ± 0.12 mg/m^3. All participants continued to work during the study. They were wearing standard work clothes and dust masks.

Blood concentrations of lead (PbB) and zinc protoporphyrin (ZPP) served as markers of lead exposure. On average, the levels of PbB and ZPP were determined every three months for the two years of observation prior entering the study. Based on the obtained values, the mean level of lead in the blood (PbB_{mean}) and the mean level of zinc protoporphyrin in the blood (ZPP_{mean}) were calculated.

The inclusion criteria were the following: occupational exposure to lead ($PbB_{mean} > 20$ µg/dl and $ZPP_{mean} > 2.5$ µg/g Hb), lack of significant findings in physical examination, and no history of any chronic disease. Exclusion criteria included contraindications for the administration of NAC.

The examined population was randomly divided into four groups. At the beginning of the study, during prophylactic medical examination, each participant of the study drew a piece of paper with the group number from a bag. Participants of the study and investigators were not blinded to the treatment group assignment. Workers in the first group (reference group) were not administered with any antioxidants, drugs, vitamins or dietary supplements, while workers in the remaining three groups were treated orally with NAC (Fluimucil®, Medagro) at three different doses for 12 weeks until the next prophylactic medical examination. Workers in the NAC 200 group were administered with 200 mg of NAC once a day, workers in the NAC 400 group were administered with 200 mg of NAC twice a day, and workers in the NAC 800 group were administered with 400 mg of NAC twice a day. At the end of the study, participants were asked to return their empty pill boxes back to one of the study investigators to assess compliance with treatment protocol.

The blood of all examined workers was drawn two times: up to three days before prophylactic medical examination at the beginning of the study and up to three days before the next prophylactic medical examination after 12 weeks of treatment or observation. The levels of PbB and ZPP were determined in the collected blood samples. The erythrocyte GSH and lipofuscin (LPS) levels and activities of glutathione reductase (GR), glutathione-S-transferase (GST) and of glucose-6-phosphate dehydrogenase (G6PD) in the blood cells were also determined.

Laboratory procedures

To obtain whole blood, erythrocytes and leukocytes, 14 ml of blood was drawn by venipuncture into tubes containing an ethylenediaminetetraacetic disodium acid (EDTA) solution as an anticoagulant.

Immediately after blood sampling, 5 ml of whole blood was centrifuged. The plasma supernatant was removed. The sedimented erythrocytes were washed three times through centrifugation with 0.9% sodium chloride solution. Subsequently, the erythrocytes were lysed with bidistilled water. Finally, 10% (v/v) hemolysate was prepared. The levels of hemoglobin, GSH and LPS and activities of GR and G6PD were measured in the hemolysate.

To isolate the leukocytes, 3 ml of the whole blood was layered over Histopaque-1077 (Sigma-Aldrich) in a 1:1 ratio and centrifuged for 30 min. Leukocytes (1.5 ml) were collected from the interface and washed three times through centrifugation with 0.9% sodium chloride solution. Finally, the lysate of leukocytes was prepared in 1.5 ml of bidistilled water. The protein level and the activities of GR, GST, and G6PD were measured in this lysate.

PbB was analyzed in the whole blood by graphite furnace atomic absorption spectrophotometry using Unicam 929 and 939OZ Atomic Absorption Spectrometers with GF90 and GF90Z Graphite Furnaces. Data were displayed as microgram per deciliter.

The level of ZPP was measured using Aviv Biomedical hematofluorometer model 206. The results were expressed in microgram ZPP per gram of hemoglobin (µg/g Hb).

The levels of GSH and LPS in erythrocytes were indicated by Pawelski[17] and Jain[18], respectively. GSH concentrations were expressed as micromoles per gram of hemoglobin (µmol/g Hb), while LPS concentrations were expressed as relative units (RU) per gram of hemoglobin (the fluorescence

Copyright © Informa Healthcare USA, Inc. 2013

of 0.1 mg/ml solution of quinidine sulfate in sulfuric acid is equal to 100 RU).

The activities of G6PD and GR in erythrocytes and leukocytes were measured according to Richterich[19] using a Shimadzu UV-1700 spectrophotometer. G6PD activity was expressed as micromoles of NADPH produced per minute normalized to 1 gram of hemoglobin in erythrocytes (IU/g Hb) and 1 gram of protein in leukocytes (IU/g P), whereas the activity of GR was expressed as micromoles of NADPH utilized per minute normalized to 1 gram of hemoglobin in erythrocytes (IU/g Hb) and 1 gram of protein in leukocytes (IU/g P). The activity of GST in leukocytes was measured according to the kinetic method of Habig and Jakoby[20] using a Shimadzu UV-1700 spectrophotometer. The activity of GST was expressed as micromoles of thioether produced per minute normalized to 1 gram of protein (IU/g P).

Statistical analysis

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

Results

At the beginning, the study consisted of 200 participants (50 subjects in each group). We further excluded three participants who had developed dyspepsia (one participant from the NAC 400 group, two participants from the NAC 800 group) and 26 participants who had not been taking NAC according to the study protocol or had not undergone the medical examination after 12 weeks of treatment. This left 49 subjects in the reference group, 40 subjects in the NAC 200 group, 44 subjects in the NAC 400 group, and 38 subjects in the NAC 800 group. Workers' age ranged between 23 and 58 years, and they were exposed to lead from 4 to 38 years.

There were no significant differences in age, years of work, body mass index (BMI), or smoking habits among the examined groups (Table 1). Similarly, there were no differences in PbB_{mean} or ZPP_{mean} values calculated before the treatment. However, the PbB levels measured before treatment were significantly higher in the NAC 400 and NAC 800 groups compared to those in the reference group. After 12 weeks of treatment, the PbB levels decreased significantly in all groups receiving NAC, while the PbB level in the reference group remained unaltered. The ZPP levels measured at the beginning of the treatment were similar in all groups. Treatment with NAC resulted in a significant decrease in ZPP levels in the NAC 200 and NAC 400 groups compared to the values before treatment. However, these alterations were not significant compared to the slight decrease in ZPP level (insignificant) observed in the reference group (Table 1). Moreover, the changes in ZPP levels were positively correlated with changes in G6PD activity in leukocytes (data not shown).

Erythrocyte GSH concentrations were significantly increased in the NAC 400 (by 5%) and NAC 800 (by 6%) groups after treatment with NAC in comparison with values at the beginning of the study. In the group of all patients treated with NAC, the GSH level increased significantly by 4%. In the NAC 800 group, the observed increase was significant compared with that of the reference group. However,

Table 1. Demographic parameters, blood lead levels (PbB), and levels of zinc protoporphyrin in the blood (ZPP) of a Pb-exposed population before and after 3 months of treatment with N-acetylcysteine (NAC) and observations in the control group.

	Control 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
PbB (μg/dl) before¶	44.1 ± 9.3	47.1 ± 8.8	0.069	44.97 ± 9.28	0.664	47.87 ± 8.49	**0.045**	48.46 ± 8.38	**0.026**
PbB (μg/dl) after§	43.2 ± 8.7	42.4 ± 9.1	0.569	40.42 ± 9.13	0.140	43.49 ± 10.12	0.896	43.14 ± 7.40	0.955
p-value*	0.981	**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**	
Δ PbB	− 0.87 ± 10.6	− 4.72 ± 7.08	**0.001**	− 4.55 ± 6.61	**0.010**	− 4.38 ± 7.44	**0.012**	− 5.32 ± 7.28	**0.004**
ZPP (μg/g Hb) before‖	8.71 ± 4.84	8.26 ± 4.07	0.542	8.51 ± 4.86	0.848	8.27 ± 3.84	0.633	8.00 ± 3.46	0.445
ZPP (μg/g Hb) after#	8.64 ± 4.74	7.36 ± 3.14	0.086	7.41 ± 3.40	0.173	7.24 ± 3.00	0.098	7.43 ± 3.08	0.177
p-value*	0.758	**< 0.001**		**0.038**		**0.005**		0.120	
Δ ZPP	− 0.07 ± 4.08	− 0.91 ± 2.73	0.120	− 1.10 ± 3.08	0.191	− 1.03 ± 2.40	0.115	− 0.57 ± 2.74	0.520

p value, comparison with the control group; p value*, comparison between obtained values before and after treatment.
†mean blood lead level before treatment, ‡mean blood zinc-protoporphyrin level before treatment, ¶blood lead level before treatment, §blood lead level after treatment, ‖ blood zinc-protoporphyrin level before treatment, and # blood zinc-protoporphyrin level after treatment.

Clinical Toxicology Downloaded from informahealthcare.com by Gazi Univ. on 08/19/14
For personal use only.

Table 2. The activities of G6PD and glutathione GR and the concentration of GSH and LPS in erythrocytes (e) in a Pb-exposed population before and after 3 months of treatment with N-acetylcysteine (NAC) and observations in the control group.

	Control 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
GSH-e concentration (μmol/g Hb) before	13.7 ± 1.68	13.3 ± 1.48	0.214	13.4 ± 0.84	0.302	13.2 ± 1.72	0.140	13.1 ± 1.70	0.160
GSH-e concentration (μmol/g Hb) after	13.8 ± 2.16	13.8 ± 2.06	0.785	13.6 ± 1.70	0.468	13.8 ± 2.24	0.932	13.9 ± 2.21	0.906
p-value*	0.651	**< 0.001**		0.467		**0.001**		**0.003**	
Δ GSH-e	0.12 ± 1.52	0.47 ± 1.49	0.089	0.12 ± 1.70	0.996	0.61 ± 1.28	***0.070***	0.69 ± 1.47	**0.047**
G6PD-e activity (IU/g Hb) before	3.49 ± 1.01	3.65 ± 0.72	0.246	3.62 ± 0.87	0.500	3.51 ± 0.62	0.894	3.83 ± 0.63	0.091
G6PD-e activity (IU/g Hb) after	3.60 ± 0.75	4.27 ± 0.84	**< 0.001**	4.50 ± 0.86	**< 0.001**	4.00 ± 0.83	**0.014**	4.35 ± 0.79	**< 0.001**
p-value*	0.560	**< 0.001**		**< 0.001**		**0.003**		**< 0.001**	
Δ G6PD-e	0.11 ± 1.26	0.62 ± 0.91	**0.003**	0.87 ± 1.03	**0.003**	0.49 ± 0.95	0.109	0.52 ± 0.69	***0.077***
GR-e activity (IU/g Hb) before	3.67 ± 1.93	3.55 ± 1.27	0.634	3.66 ± 1.52	0.988	3.48 ± 1.27	0.588	3.50 ± 0.99	0.635
GR-e activity (IU/g Hb) after	3.37 ± 1.47	3.60 ± 1.14	0.291	3.49 ± 1.39	0.698	3.55 ± 1.06	0.507	3.76 ± 0.93	**0.047**
p-value*	0.635	0.390		0.697		0.657		0.115	
Δ GR-e	− 0.29 ± 1.82	0.05 ± 1.35	0.178	− 0.17 ± 1.52	0.729	0.07 ± 1.40	0.284	0.25 ± 1.08	0.105
LPS-e concentration (RU/g Hb) before	2.27 ± 0.50	2.48 ± 0.52	**0.018**	2.41 ± 0.42	0.164	2.47 ± 0.58	0.080	2.56 ± 0.55	**0.012**
LPS-e concentration (RU/g Hb) after	2.46 ± 0.49	2.21 ± 0.45	**0.001**	2.29 ± 0.40	0.071	2.11 ± 0.50	**0.001**	2.23 ± 0.43	**0.015**
p-value*	**0.001**	**< 0.001**		**0.032**		**< 0.001**		**0.002**	
Δ LPS-e	0.19 ± 0.45	− 0.27 ± 0.49	**< 0.001**	− 0.12 ± 0.35	**< 0.001**	− 0.35 ± 0.47	**< 0.001**	− 0.33 ± 0.61	**< 0.001**

p value, in comparison with the control group; p value*, comparison between after and before treatment.

Clinical Toxicology Downloaded from informahealthcare.com by Gazi Univ. on 08/19/14
For personal use only.

compared with the NAC 400 group and the reference group, the observed changes in GSH levels showed a strong tendency to be significantly different (Table 2).

The activities of erythrocyte G6PD were significantly increased in all groups receiving NAC in comparison with values at the beginning of the study (by 24% in the NAC 200 group, by 14% in the NAC 400 and 800 groups, and significantly by 17% in the group of all patients treated with NAC) (Table 2).

There were no significant differences in G6PD activity in leukocytes or GR activity in leukocytes and erythrocytes in examined groups before and after treatment. The GST activity in leukocytes decreased significantly or showed a tendency toward a decrease after treatment in these groups (by 34% in the NAC 200 group, by 21% in the NAC 400 group, by 22% in the NAC 800 group, and significantly by 24% in the group of all patients treated with NAC). The observed changes were also significant compared to the slight increase in GST activity observed in the reference group (Tables 2 and 3).

The erythrocyte LPS level before treatment was significantly higher in the NAC 800 group than in the reference group. After treatment, the LPS level decreased significantly in all examined groups compared with that in the beginning of the study (by 5% in the NAC 200 group, by 15% in the NAC 400 group, by 13% in the NAC 800 group and significantly by 11% in the group of all patients treated with NAC) and the reference group (Table 2).

Discussion

There have only been a few studies in vivo and in vitro on animals that investigated the therapeutic effect of NAC in lead poisoning. Therefore, the results of our findings in humans are difficult to compare.

In the present study, NAC increased erythrocyte GSH levels in a dose-dependent manner (a significant effect was observed only after the administration of 400 and 800 mg). The effect of NAC administration on LPS levels was also dose dependent. Because LPS is formed by cross-linked aggregates of oxidized proteins and lipids,[21] its level reflects oxidative damage to not only lipids, such as malondialdehyde (MDA), but also proteins. An increased level of LPS by 259% was reported in our previous study[22] conducted on lead-exposed workers (mean PbB = 40.53 ± 2.47 μg/dl).

Considering the elevated GSH levels and decreased LPS levels indicated after 12 weeks of NAC treatment, it is possible to state that NAC reduces the intensity of lead-induced oxidative stress. The results of studies conducted on animals support our study. In lead-exposed rats, NAC administration restored depleted GSH content and decreased elevated MDA and GSSG levels in the blood, lymphocytes, liver, kidney, and brain.[13–16]

GSH is a tripeptide composed of glutamate, cysteine, and glycine. GSH acts as an antioxidant because of the thiol moiety of the cysteine residue. GSH primarily acts as a direct scavenger of ROS in a non-enzymatic manner and

Copyright © Informa Healthcare USA, Inc. 2013

Table 3. The activities of G6PD, GR, and GST in leukocytes (l) in a Pb-exposed population before and after 3 months of treatment with N-acetylcysteine (NAC) and observations in the control group.

	Control 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
G6PD-l activity (IU/g protein) before	43.4 ± 11.3	39.4 ± 14.1	0.307	44.9 ± 12.7	0.743	38.4 ± 13.9	0.225	38.1 ± 14.8	0.225
G6PD-l activity (IU/g protein) after	44.8 ± 12.1	42.2 ± 15.9	0.553	40.4 ± 13.8	0.366	43.8 ± 18.7	0.855	41.3 ± 13.6	0.404
p-value*	0.594	0.265		0.470		0.238		0.371	
Δ G6PD-l	1.42 ± 11.6	3.52 ± 18.6	0.675	− 0.84 ± 6.79	0.544	5.48 ± 21.3	0.494	3.26 ± 18.9	0.733
GR-l activity (IU/g protein) before	33.0 ± 14.5	29.7 ± 13.0	0.376	29.5 ± 15.0	0.531	30.4 ± 14.07	0.563	29.0 ± 10.9	0.304
GR-l activity (IU/g protein) after	34.8 ± 21.4	28.66 ± 10.6	0.095	27.1 ± 10.3	0.230	28.0 ± 8.97	0.129	30.1 ± 12.4	0.351
p-value*	0.730	0.620		0.875		0.695		0.750	
Δ GR-l	1.79 ± 20.1	− 1.19 ± 16.2	0.534	− 3.22 ± 16.1	0.466	− 2.41 ± 17.7	0.471	1.08 ± 14.8	0.893
GST-l activity (IU/g protein) before	10.17 ± 1.89	10.18 ± 7.28	0.998	11.02 ± 3.59	0.429	10.25 ± 7.77	0.969	9.70 ± 8.12	0.827
GST-l activity (IU/g protein) after	11.33 ± 2.08	7.72 ± 3.12	**< 0.001**	7.30 ± 2.66	**< 0.001**	8.05 ± 3.51	**0.002**	7.55 ± 2.92	**< 0.001**
p-value*	0.124	**< 0.001**		**0.009**		0.084		***0.054***	
Δ GST -l	1.16 ± 2.87	− 2.26 ± 7.03	**0.007**	− 2.63 ± 3.93	**0.003**	− 2.20 ± 7.29	**0.039**	− 2.15 ± 7.99	**0.021**

p value, in comparison with the control group; p value*, comparison between after and before treatment.

Clinical Toxicology Downloaded from informahealthcare.com by Gazi Univ. on 08/19/14
For personal use only.

a co-factor for glutathione peroxidase (GPx), which reduces hydrogen peroxide and other peroxide compounds. GPx oxidizes GSH, resulting in the generation of glutathione disulfide (GSSG). GSSG is reduced to GSH by GR. In this reaction, NADPH serves as an electron donor. Consequently, GR activity and the GSH/GSSG ratio are sensitive to cellular NADPH levels.[23] In mature erythrocytes, the main source of NADPH is the pentose phosphate pathway, which generates most of the extra-mitochondrial NADPH through the oxidation of glucose-6-phosphate by G6PD.[24,25]

Because thiol groups are essential for the catalytic activity of G6PD, it is reasonable to expect that its activity decreases in lead poisoning. This hypothesis is supported by a study by Lachant et al.,[26] who investigated the influence of lead acetate on human erythrocyte metabolism in vitro and suggested lead-induced reversible suppression of pentose phosphate pathway function due to a decrease in G6PD activity. By contrast, Gurer-Orhan et al.[27] observed significantly elevated G6PD activity in lead-exposed workers (mean PbB = 54.6 ± 17 μg/dl). In our previous study,[28] significantly elevated G6PD activity in lead-exposed workers (mean PbB = 40.6 ± 6.7 μg/dl) was also reported. This inconsistency may be because a decreased NADPH/$NADP^+$ ratio under lead-induced oxidative stress conditions may elevate G6PD activity despite its lead-mediated direct suppression.[24,25,29] Therefore, it is possible to state that the influence of lead on G6PD activity could be different and most likely depends primarily on the lead concentration and the duration of lead exposure.

In the present study, we showed that NAC administration elevated G6PD activity in erythrocytes. One possible mechanism of this beneficial effect of applied treatment is that thiol groups of NAC compete with thiol groups within G6PD to bind to lead ions. As a result, the formation of lead–thiol complexes within G6PD could be reduced.

At the same time, we observed no changes in G6PD activity in leukocytes. This difference between erythrocytes and leukocytes is most likely caused by the fact that erythrocytes have less diverse sources of NADPH[24,25] and are more sensitive to oxidative stress. They are exposed to a higher concentration of oxygen and contain hemoglobin, which undergoes auto-oxidation.[30] Moreover, erythrocytes are exposed to delta-aminolevulinic acid (ALA), which accumulates in lead poisoning and acts as a pro-oxidant.[11]

In our previous study,[31] we showed that GR activities were unchanged in a similar group of lead-exposed workers compared to the unexposed controls. Unaltered GR activity after NAC administration, which was also shown in the present study, confirms the hypothesis that the influence of lead poisoning on the activity of this enzyme is limited.

In addition to being a cofactor of GPx, GSH is involved in the detoxification of numerous agents. The GST family of enzymes catalyzes the formation of GSH–xenobiotic conjugates that are more water soluble than the parent compound and easier to excrete.[23] Some authors have indicated that lead induces GST activity. Elevated GST activity in the rat kidney was reported by Conterato et al.[32] and Moser et al.[33] Consistently, elevated GST activities were observed in the rat brain[34] and the liver, heart and brain of chick embryos.[35] These animal studies are in accordance with our previous research,[36] in which we reported increased GST activity in lead-exposed workers (PbB = 25–45 μg/dl). Consistently, Conterato et al.[37] reported significantly increased GST activity in painters (mean PbB = 5.4 μg/dl) and battery workers (mean PbB = 49.8 μg/dl). Other studies have indicated that lead decreases GST activity in rats.[38,39] Tsai et al.[40] postulate

that GSH depletion upregulates the expression of the pi class of GST (GSTP). In that study, NAC and GSH monoethyl ester were able to reverse the induction of GSTP. The findings of Tsai et al.[40] are in accordance with the present study because we showed that after NAC treatment, the activities of leukocyte GST were significantly decreased or showed a tendency toward lower values.

The results from this study need to be evaluated within the context of its limitations. A major limitation was the fact that subjects were all men. Besides, participants of the study were not blinded to treatment. The next limitation was the difficulty to verify that the NAC had been actually taken by the workers in the doses stated. Furthermore, only biochemical outcomes were measured.

Conclusions

The administration of NAC decreases oxidative stress in workers exposed to lead in a dose-dependent manner. NAC has antioxidant capacity for lead via stimulating GSH synthesis. The influence of NAC on GSH-related enzymes is complex and results in increased NADPH production and lower utilization of GSH by GST.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

Funding

The Ministry of Science and Higher Education and the Medical University of Silesia supported this work.

References

1. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates. Interdiscip Toxicol 2012; 5:47–58.
2. Patrick L. Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. Altern Med Rev 2006; 11:2–22.
3. Caylak E, Aytekin M, Halifeoglu I. Antioxidant effects of methionine, alpha-lipoic acid, N-acetylcysteine and homocysteine on lead-induced oxidative stress to erythrocytes in rats. Exp Toxicol Pathol 2008; 60:289–294.
4. Patrick L. Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Altern Med Rev 2006; 11:114–127.
5. Wang L, Wang Z, Liu J. Protective effect of N-acetylcysteine on experimental chronic lead nephrotoxicity in immature female rats. Hum Exp Toxicol 2010; 29:581–591.
6. Nehru B, Kanwar SS. Modulation by N-acetylcysteine of lead-induced alterations in rat brain: reduced glutathione levels and morphology. Toxicol Mech Methods 2007; 17:289–293.
7. Sadowska AM, Manuel-Y-Keenoy B, De Backer WA. Antioxidant and anti-inflammatory efficacy of NAC in the treatment of COPD: discordant in vitro and in vivo dose-effects: a review. Pulm Pharmacol Ther 2007; 20:9–22.
8. Chen W, Ercal N, Huynh T, Volkov A, Chusuei CC. Characterizing N-acetylcysteine (NAC) and N-acetylcysteine amide (NACA) binding for lead poisoning treatment. J Colloid Interface Sci 2012; 371:144–149.
9. Benrahmoune M, Thérond P, Abedinzadeh Z. The reaction of superoxide radical with N-acetylcysteine. Free Radic Biol Med 2000; 29:775–782.
10. Gillissen A, Schärling B, Jaworska M, Bartling A, Rasche K, Schultze-Werninghaus G. Oxidant scavenger function of ambroxol in vitro: a comparison with N-acetylcysteine. Res Exp Med (Berl) 1997; 196:389–398.
11. Ercal N, Treeratphan P, Hammond TC, Matthews RH, Grannemann NH, Spitz DR. In vivo indices of oxidative stress in lead-exposed C57BL/6 mice are reduced by treatment with meso-2,3-dimercaptosuccinic acid or N-acetylcysteine. Free Radic Biol Med 1996; 21: 157–161.
12. Aykin-Burns N, Franklin EA, Ercal N. Effects of N-acetylcysteine on lead-exposed PC-12 cells. Arch Environ Contam Toxicol 2005; 49:119–123.
13. Ercal N, Neal R, Treeratphan P, Lutz PM, Hammond TC, Dennery PA, Spitz DR. A role for oxidative stress in suppressing serum immunoglobulin levels in lead-exposed Fisher 344 rats. Arch Environ Contam Toxicol 2000; 39:251–256.
14. Nehru B, Kanwar SS. N-acetylcysteine exposure on lead-induced lipid peroxidative damage and oxidative defense system in brain regions of rats. Biol Trace Elem Res 2004; 101:257–264.
15. Pande M, Mehta A, Pant BP, Flora SJ. Combined administration of a chelating agent and an antioxidant in the prevention and treatment of acute lead intoxication in rats. Environ Toxicol Pharmacol 2001; 9:173–184.
16. Tandon SK, Singh S, Prasad S, Srivastava S, Siddiqui MK. Reversal of lead-induced oxidative stress by chelating agent, antioxidant, or their combination in the rat. Environ Res 2002; 90:61–66.
17. Pawelski S. Diagnostyka laboratoryjna w hematologii. PZWL, Warszawa; 1983.
18. Jain SK. In vivo externalization of phosphatidylserine and phosphatidylethanolamine in the membrane bilayer and hypercoagulability by the lipid peroxidation of erythrocytes in rats. J Clin Invest 1985; 76:281–286.
19. Richterich R. Chemia kliniczna. Warszawa: PZWL; 1971.
20. Habig WH, Jakoby WB. Assays for differentiation of glutathione S-transferases. Methods Enzymol 1981; 77:398–405.
21. Jung T, Bader N, Grune T. Lipofuscin: formation, distribution, and metabolic consequences. Ann N Y Acad Sci 2007; 1119:97–111.
22. Kasperczyk A, Słowińska-Łożyńska L, Dobrakowski M, Zalejska-Fiolka J, Kasperczyk S. The effect of lead-induced oxidative stress on blood viscosity and rheological properties of erythrocytes in lead exposed humans. Clin Hemorheol Microcirc 2013 [Epub ahead of print] doi:10.3233/CH-131678.
23. Backos DS, Franklin CC, Reigan P. The role of glutathione in brain tumor drug resistance. Biochem Pharmacol 2012; 83:1005–1012.
24. Cocco P. Occupational lead exposure and screening of glucose-6-phosphate dehydrogenase polymorphism: useful prevention or nonvoluntary discrimination? Int Arch Occup Environ Health 1998; 71:148–150.
25. Gurer H, Ercal N. Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic Biol Med 2000; 29:927–945.
26. Lachant NA, Tomoda A, Tanaka KR. Inhibition of the pentose phosphate shunt by lead: a potential mechanism for hemolysis in lead poisoning. Blood 1984; 63:518–524.
27. Gurer-Orhan H, Sabir HU, Ozgüneş H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. Toxicology 2004; 195:147–154.
28. Kasperczyk A, Dobrakowski M, Ostałowska A, Zalejska-Fiolka J, Birkner E. The metabolism of carbohydrates and lipid peroxidation in lead exposed workers. Toxicol Ind Health 2013. [Epub ahead of print] doi:10.1177/0960327112468177.
29. Mudipalli A. Lead hepatotoxicity & potential health effects. Indian J Med Res 2007; 126:518–527.
30. Gürer H, Ozgünes H, Neal R, Spitz DR, Erçal N. Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. Toxicology 1998; 128:181–189.

Clinical Toxicology Downloaded from informahealthcare.com by Gazi Univ. on 08/19/14
For personal use only.

Copyright © Informa Healthcare USA, Inc. 2013

31. Kasperczyk S, Kasperczyk A, Ostalowska A, Dziwisz M, Birkner E. Activity of glutathione peroxidase, glutathione reductase, and lipid peroxidation in erythrocytes in workers exposed to lead. Biol Trace Elem Res 2004;102:61–72.
32. Conterato GM, Augusti PR, Somacal S, Einsfeld L, Sobieski R, Torres JR, Emanuelli T. Effect of lead acetate on cytosolic thioredoxin reductase activity and oxidative stress parameters in rat kidneys. Basic Clin Pharmacol Toxicol 2007; 101:96–100.
33. Moser R, Oberley TD, Daggett DA, Friedman AL, Johnson JA, Siegel FL. Effects of lead administration on developing rat kidney. I. Glutathione S-transferase isoenzymes. Toxicol Appl Pharmacol 1995; 131:85–93.
34. Saxena G, Flora SJ. Lead-induced oxidative stress and hematological alterations and their response to combined administration of calcium disodium EDTA with a thiol chelator in rats. J Biochem Mol Toxicol 2004; 18:221–233.
35. Somashekaraiah BV, Padmaja K, Prasad AR. Lead-induced lipid peroxidation and antioxidant defense components of developing chick embryos. Free Radic Biol Med 1992; 13:107–114.
36. Kasperczyk S, Kasperczyk A, Birkner E, Ostałowska A, Dziwisz M. [Activity of glutathione S-transferase in erythrocytes of workers exposed to lead]. Bromat Chem Toksykol 2002; 35:173–178.
37. Conterato GM, Bulcão RP, Sobieski R, Moro AM, Charão MF, de Freitas FA, et al. Blood thioredoxin reductase activity, oxidative stress and hematological parameters in painters and battery workers: relationship with lead and cadmium levels in blood. J Appl Toxicol 2013; 33:142–150.
38. Jackie T, Haleagrahara N, Chakravarthi S. Antioxidant effects of Etlingera elatior flower extract against lead acetate – induced perturbations in free radical scavenging enzymes and lipid peroxidation in rats. BMC Res Notes 2011; 4:67.
39. Mehana EE, Meki AR, Fazili KM. Ameliorated effects of green tea extract on lead induced liver toxicity in rats. Exp Toxicol Pathol 2012; 64:291–295.
40. Tsai CW, Lin AH, Wang TS, Liu KL, Chen HW, Lii CK. Methionine restriction up-regulates the expression of the pi class of glutathione S-transferase partially via the extracellular signal-regulated kinase-activator protein-1 signaling pathway initiated by glutathione depletion. Mol Nutr Food Res 2010; 54:841–850.

Clinical Toxicology Downloaded from informahealthcare.com by Gazi Univ. on 08/19/14
For personal use only.