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The Comparison of Antioxidant and Hematological Properties of N-Acetylcysteine and α-Lipoic Acid in Physically Active Males

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Summary

The aim of this study was to follow up whether the modification of pro-antioxidant status by oral thiol administration such as N-acetylcysteine and α-lipoic acid affects the hematological response. Twenty-eight healthy men participated in two independent experiments. Subjects were randomly assigned to one of four groups: controls (C_{NAC} and C_{ALA}), N-acetylcysteine (NAC) and α-lipoic acid (ALA). 1200 mg of N-acetylcysteine, 600 mg of α-lipoic acid or placebo were administered for 8 days in two doses. NAC or ALA administration significantly elevated plasma total antioxidant status (TAS) and reduced protein carbonylation (PC) and lipid peroxidation (TBARS) by more than 30 %. The reduced glutathione (GSH) and hematological parameters changed only in response to NAC administration. NAC significantly elevated the level of GSH (+33 %), EPO (+26 %), Hb (+9 %) and Hct (+9 %) compared with C_{NAC}. The mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) also increased by more than 12 % after NAC. The numerous negative or positive correlations between the measures of TAS, PC, TBARS and hematological parameters were found, which suggest the NAC-induced interaction between pro-antioxidant and hematological values. Our study has shown that both N-acetylcysteine and α-lipoic acid intake reveal an antioxidant action, but only N-acetylcysteine improves the haematological response.

Key words

Thiol • Oxidative damage • Antioxidant • Erythropoietin

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Introduction

The thiols such as cysteine derivatives, glutathione, lipoic acid and ergothioneine are exceptional compounds involved in production of reactive oxygen species (ROS), which contributes to gene expression, proliferation, antioxidant defense, erythropoiesis, immunological response etc. (Sen and Packer 2000, Valko *et al.* 2007).

N-acetylcysteine (NAC) and α-lipoic acid (ALA) as the pro-glutathione dietary supplements have been subject of intensive research in nutrition in the last few years. Both compounds have been suggested to function as powerful antioxidants. NAC and ALA have shown the ability to react directly with ROS such as hydroxyl radical, hypochlorous acid and singlet oxygen, and indirectly through the reduction of glutathione disulphide, tocopherol radicals and ascorbate. NAC and ALA can work as a redox regulator of myoglobin, prolactin, thioredoxin, glucose transporter protein (GLUT4) and transcription factors such as NF-κB, AP-1

and HIF-1. Moreover, ALA as lipoamid, has functioned as a cofactor in the multienzyme complexes that catalyse the oxidative decarboxylation of α-keto acids such as pyruvate, α-ketoglutarate, and branched chain α-keto acids (Sen and Packer 2000, Moini *et al.* 2002, Cakatay 2005, Kerksick and Willoughby 2005, Valko *et al.* 2007).

Although N-acetylcysteine and α-lipoic acid have been commonly used remedies, their use by physically active persons as a pro-glutathione dietary supplement gives rise to a certain controversy. Firstly, it has been observed that NAC or ALA supplementation prevented the decline of thiol content in muscle, lung and blood, and weakened oxidative damage (Sen *et al.* 1994, Moini *et al.* 2002). On the other hand, it has been shown that long-term NAC or ALA administration led to enhancement of lipid peroxidation, mitochondrial damage and inhibition of glycogen synthesis (Kleinveld *et al.* 1992, Childs *et al.* 2001, Moini *et al.* 2002, Cakatay 2005). Secondly, NAC has been marked as a useful compound to regulate the differentiation of erythroid progenitors (Nagata *et al.* 2007). It has also been helpful in increasing the plasma EPO concentration in humans before and during hypoxia (Hildebrandt *et al.* 2002). In another study, NAC and ALA did not demonstrate any modulatory effect on EPO production under hypoxia conditions (Freudenthaler *et al.* 2002).

Due to the interesting role of thiols in cell metabolism and contradictory information concerning NAC and ALA, the aim of this study was to determine 1) whether an improvement of antioxidant status and reduction of oxidative damage by oral N-acetylcysteine and α-lipoic acid administration equally affect the hematological response, and 2) whether there is a direct relationship between total antioxidant status and erythropoietin (EPO) secretion.

Methods

Twenty-eight healthy trained males with forced training experience of at least 3 years, physical education students, participated in the randomized, double-blind, placebo-controlled and cross-over studies (Table 1). All the subjects were informed of the aim of the study and were given their written consent for participation in the project. The protocol of the study was approved by the local ethics committee in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

1200 mg of N-acetylcysteine (Hexal AG Germany) – NAC group, 600 mg of α-lipoic acid (Wörwag Pharma Germany) – ALA group or 700 mg of lactose (placebo) – C_{NAC} and C_{ALA} groups were administered for eight days in two doses as powder dissolved in 50 ml of water. The participants took the first dose in the morning in a fasted state and the second one 2 h before an evening meal. The wash-out period between the trials with thiol compounds and placebo was three weeks. Subjects had not taken any antioxidants supplements (vitamins or minerals) for 4 weeks prior to the study. During the 8-day experimental period, the athletes participated in training according the training program. One day before blood sampling, the subjects did not perform any strenuous exercises.

Table 1. Physical characteristics of subjects.

Group	Age (years)	Body mass (kg)	Height (cm)	Body fat (%)
C_{NAC}/*NAC*	20.3	83.4	178.6	16.5
(n = 15)	± 2.3	± 14.4	± 8.5	± 4.5
C_{ALA}/*ALA*	25.5	86.4	180.5	14.1
(n = 13)	± 6.0	± 8.1	± 5.9	± 3.9

Blood samples were obtained from an antecubital forearm vein with an anticoagulant ($EDTAK_2$) in the morning in a fasted state. The samples were immediately placed in 4 °C after collection. Within 10 min, the blood samples were centrifuged at 2500 x g and 4 °C for 10 min. Aliquots of plasma were stored at –20 °C. All samples were analyzed within 7 days.

Total antioxidant status (TAS) of the plasma was measured using method developed by Randox laboratories (UK). The method is based on the formation of 2'-2'-azino-di-[3-ethylbenzthiazoline sulphonate] radical which is measured spectrophotometrically at 600 nm. Detection limit for the TAS kit was 0.21 mmol/l and the intra-assay coefficient of variation was 2.77 %.

Plasma protein carbonyls (PC) were measured according to Levine *et al.* (1990) using 2,4-dinitrophenyl hydrazine. The carbonyl content was calculated using extinction coefficient of 22000 $M^{-1} \cdot l^{-1} \cdot cm^{-1}$ and expressed as nmol PC per mg of plasma protein. Protein concentration was determined by the method of Bradford (1976).

Plasma lipid peroxidation products were estimated using the measurement of thiobarbituric acid-reactive substance (TBARS) level according to the method of Buege and Aust (1991). The TBARS level

was expressed as nmol of malondialdehyde using 1,1,3,3- tetraethoxypropane as a standard. TBARS detection limit was 0.13 nmol/ml. The intra-assay coefficients of variation for PC and TBARS procedures were less than 10 %.

Blood reduced glutathione concentration was estimated by the method of Beutler *et al.* (1963) using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). GSH detection limit for the procedure was 2.5 μg/ml and the intra-assay coefficient of variation was <10 %.

The plasma EPO level was measured using immunoassay system Immulite 2000 DPC (USA). Detection limit for the EPO assay was 0.24 mU/ml and the intra-assay coefficient of variation was 5.9 %.

Hemoglobin (Hb), hematocrit (Hct), erythrocytes (RBC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were assessed using ABX Micros OT 16 (France).

Statistics

The results are expressed as mean ± S.D., and statistical analysis was carried out by one-way ANOVA. The *post-hoc* Tukey's test for multiple comparisons among means was used to compare intergroup differences. Correlations were calculated by the Pearson correlation coefficients. $P<0.05$ was accepted as significant.

Results

As shown in Figures 1-3, the administration of NAC or ALA significantly improved plasma antioxidant defense and reduced oxidative damage. Total antioxidant status was significantly elevated by both NAC and ALA. In NAC group, an increase in total antioxidant status was 38 % compared with C_{NAC}, and in ALA group was 9 % compared with C_{ALA}. This has indicated that the influence of N-acetylcysteine on total antioxidant status was fourfold higher than α-lipoic acid (Fig. 1). NAC and ALA revealed a distinct antioxidant action in relation to plasma protein carbonylation and lipid peroxidation products. NAC or ALA administration induced significant decrease in PC by 28 % compared with C_{NAC} and C_{ALA} (Fig. 2). TBARS concentration reached almost 40 % declines in NAC and ALA groups in relation to controls (Fig. 3). In NAC group, TAS indirectly correlated with PC ($r = –0.746$, $P<0.001$) and TBARS ($r = –0.562$, $P<0.01$). In ALA group, the values of correlation coefficients were smaller, i.e. $r = –0.402$ ($P<0.05$) for TAS and PC, and $r = –0.456$ ($P<0.05$) for TAS and TBARS.

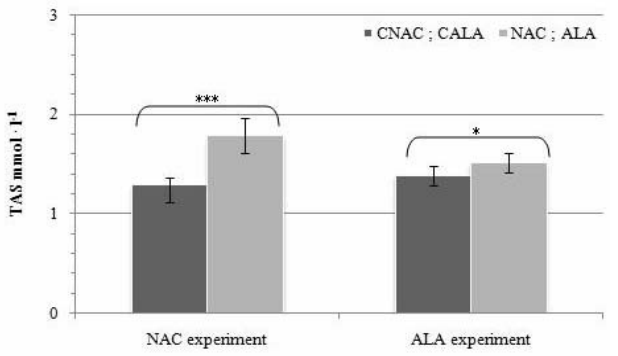

Fig. 1. Post-intervention changes in total antioxidant status (TAS); CNAC and CALA – placebo; NAC – N-acetylcysteine, ALA – α-lipoic acid; *P<0.05, **P<0.01 and ***P<0.001 indicate statistical differences between controls and supplemented groups.

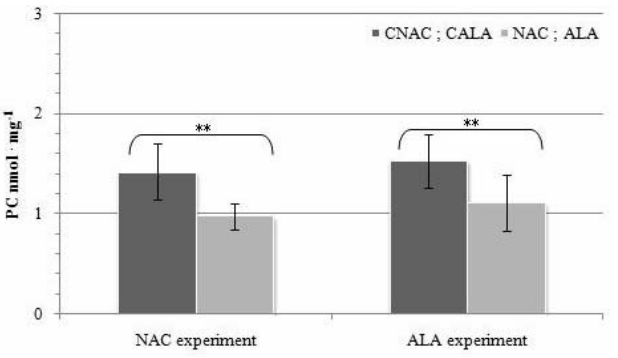

Fig. 2. Post-intervention changes in protein carbonylation products (PC); CNAC and CALA – placebo; NAC – N-acetylcysteine, ALA – α-lipoic acid; * P<0.05, ** P<0.01 and *** P<0.001 indicate statistical differences between controls and supplemented groups.

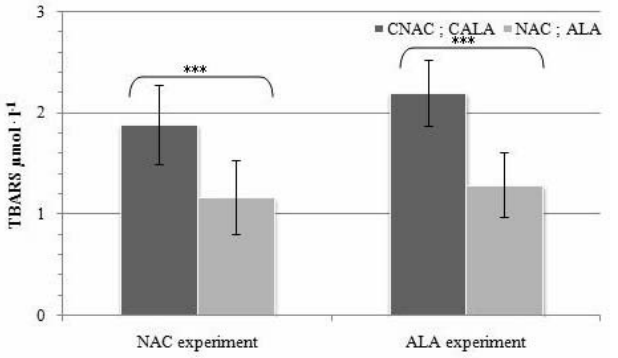

Fig. 3. Post-intervention changes in lipid peroxidation products (TBARS); CNAC and CALA – placebo; NAC – N-acetylcysteine, ALA – α-lipoic acid; * P<0.05, ** P<0.01 and *** P<0.001 indicate statistical differences between controls and supplemented groups.

Table 2. The post-intervention levels of reduced glutathione (GSH) and hematological parameters.

Parameter	C_{NAC}	NAC	C_{NAC} vs. NAC	C_{ALA}	ALA	C_{ALA} vs. ALA
GSH (mg/ g Hb)	1.54 ± 0.25	2.05 ± 0.25	P<0.01	1.94 ± 0.18	2.00 ± 0.22	ns
EPO (U/ l)	8.91 ± 1.95	11.23 ± 3.02	P<0.01	7.42 ± 1.70	8.81 ± 2.04	ns
Hb (g/dl)	14.51 ± 0.72	16.00 ± 0.93	P<0.001	14.85 ± 0.80	14.39 ± 0.75	ns
Hct (%)	42.51 ± 2.67	46.55 ± 2.87	P<0.01	42.07 ± 2.44	42.22 ± 2.11	ns
RBC ($10^6/\mu l$)	5.42 ± 0.34	5.29 ± 0.38	ns	5.27 ± 0.31	5.16 ± 0.24	ns
MCV (fl)	78.50 ± 2.10	88.07 ± 2.63	P<0.001	80.00 ± 3.08	80.00 ± 3.81	ns
MCH (pg/cell)	26.84 ± 1.16	30.29 ± 0.90	P<0.001	28.21 ± 1.18	27.93 ± 1.45	ns

C_{NAC} and C_{ALA} – controls; NAC – N-acetylcysteine, ALA – α-lipoic acid; C_{NAC} vs. NAC and C_{ALA} vs. ALA indicate statistical differences between control and supplemented groups.

Table 3. The relationships between total antioxidant status and hematological parameters observed in study with N-acetylcysteine.

	EPO	Hb	Hct	RBC	MCV	MCH
TAS	r = 0.448*	r = 0.637*	r = 0.565*	r = –0.177	r = 0.841*	r = 0.753*
	P<0.05	P<0.01	P<0.01	P>0.05	P<0.001	P<0.001
PC	r = –0.520*	r = –0.499*	r = –0.446*	r = 0.111	r = –0.660*	r = –0.569*
	P<0.01	P<0.05	P<0.05	P>0.05	P<0.001	P<0.01
TBARS	r = –0.368	r = –0.622*	r = –0.448*	r = –0.108	r = –0.369	r = –0.436*
	P>0.05	P<0.001	P<0.05	P>0.05	P>0.05	P<0.05

* P<0.05.

NAC administration remarkably affected reduced glutathione concentration. NAC elevated GSH level by 33 % compared with C_{NAC}, whereas ALA did not cause any changes in GSH (Table 2). In NAC group, reduced glutathione concentration directly correlated with TAS (r = 0.549, P<0.01).

Haematological parameters markedly responded to NAC administration (Table 2). NAC significantly elevated the plasma of EPO (+26 %), Hb (+9 %) and Hct (+9 %) compared with C_{NAC}. The mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) increased by 12 % following NAC, whereas erythrocyte count did not change in relation to C_{NAC}. The numerous negative or positive correlations between the measures of antioxidant status, oxidative damage markers (PC and TBARS) and hematological parameters were found, which suggest the NAC-induced interaction between pro-antioxidant and hematological response (Table 3). ALA administration did not induce any changes in levels of EPO, Hb, Hct, RBC, MCV and MCH, and any relationships between antioxidant status and hematological response.

Discussion

The main purpose of the present study was to determine the effects of eight-day thiol compounds administration on plasma pro-antioxidant status and hematological response, and the possible relationship between antioxidant status and EPO level. Both NAC and ALA administration elevated plasma total antioxidant status, but an increase in TAS was 4-fold higher in NAC than in ALA group. This could be related to fast and active transport of NAC into cells, its deacylation and use in glutathione synthesis and then the releasing of cysteine or glutathione from cells to plasma. Nielsen *et al.* (2001) have demonstrated a high cysteine level in plasma after three-day NAC supplementation (6 g/day) in athletes. Medved *et al.* (2003) have shown that pre-exercise N-acetylcysteine infusion increased blood glutathione during sprint test and recovery. Contrary to NAC, ALA has been an autonomous element of plasma total antioxidant status, not as a substrate to glutathione synthesis. ALA can increase GSH concentration only through reduction of disulphide glutathione and increase

in cystine utilization (Han *et al.* 1997).

The study has demonstrated two markers of oxidative damage, the carbonyl groups (PC) and thiobarbituric acid-reactive substances (TBARS). Although the TBARS and PC have been considered as non-specific techniques, using them can offer an empirical view on the complex process of lipid peroxidation and protein carbonylation. The reduction of peroxidation and carbonylation, followed by thiol compounds, was observed by many authors (Hagen *et al.* 2002, Niess *et al.* 2004, Marsh *et al.* 2006, Ates *et al.* 2008). In present study, NAC or ALA administration markedly declined the plasma PC and TBARS levels compared with controls. Moreover, both markers of oxidative damage indirectly correlated with total antioxidant status. It means that thiol supplementation significantly enhances antioxidant defense and reduces oxidative damage in tissues. This also points out that the applied doses of NAC and ALA are optimal and did not induce lipid peroxidation. Kleinveld *et al.* (1992), Childs *et al.* (2001) and Moini *et al.* (2002) have demonstrated temporal increase in peroxidation after NAC or ALA administration. The excess of thiols can cause the thiol auto-oxidation which is a potential source of reactive oxidants and may contribute to the cytotoxicity of reactive thiols such as cysteine and cysteamine (Winterbourn *et al.* 2002). According to Winterbourn *et al.* (2002) the auto-oxidation of thiols is catalyzed by superoxide dismutase and can be enhanced by iron and other transition metal ions. Furthermore, the reaction can be additionally reinforced by vitamin C with ensuing ROS production. Presumably, vitamin C was the main reason for the 30 % increase in plasma lipid peroxidation in the study performed by Childs *et al.* (2001). These authors applied the standard dose of NAC (10 mg/kg) and standard period of supplementation (7 days) but used high dose of vitamin C (12.5 mg/kg). Therefore, in our opinion a long-term and high thiol intake with compounds that can generate reactive oxidants should not be recommended to physically active persons.

Contrary to expectations, only NAC administration resulted in an elevation of the GSH concentration. This result is similar to other described in the literature. Medved *et al.* (2003) have observed that intravenous NAC infusion increased GSH and decreased GSSG levels. Matuszczak *et al.* (2005) have reported that oral NAC intake caused an increase in erythrocyte GSH and plasma cysteine. Formerly, Sen *et al.* (1994) have found that treatment with 800 mg NAC for three days diminished blood glutathione oxidation and lipid peroxidation, and also increased reduced glutathione and enhanced the net peroxyl radical scavenging capacity of the plasma.

The most interesting results of our study have been the positive correlations between plasma total antioxidant status and hematological response in subjects receiving NAC but not ALA. Recently, it has been shown that EPO production and erythroid differentiation are regulated by ROS, especially H_2O_2, which are involved in redox-sensitive signaling pathways through down-regulation of transcription factors (Fandrey *et al.* 1994, Huang *et al.* 1996, Nagata *et al.* 2007). This means that ROS generation can suppress EPO synthesis, whereas antioxidants can stimulate its synthesis. Hildebrandt *et al.* (2002) were the first to demonstrate that EPO secretion may be modulated by exogenous thiols such as NAC. Our results have confirmed the suggestions concerning the relationship between oxidative stress and erythropoiesis and have suggested that only some antioxidants could modulate hematological response.

Earlier, the other researchers made an attempt to evaluate an effect of different antioxidants, such as β-carotene, desferrioxamine, tea polyphenols, α-tocopherol, ascorbic acid, reduced glutathione, N-acetylcysteine and α-lipoic acid, on hematological alterations or EPO gene expression under normoxia or hypoxia conditions (Jelkmann *et al.* 1997, Freudenthaler *et al.* 2002, Hildebrandt *et al.* 2002, Niess *et al.* 2004, Senturk *et al.* 2005, Zhang *et al.* 2006). Our study has shown that only NAC modulated the hematological response, whereas ALA administration did not induce any changes in EPO levels and other hematological parameters. Moreover, ALA did not also increase GSH concentration, although it influenced on total antioxidant status and oxidative damage markers. This has suggested that reduced glutathione could be the main compound linking the ROS and EPO production.

In summary, eight-day administration with 1200 mg N-acetylcysteine or 600 mg α-lipoic acid 1) led to the improvement of total antioxidant status and the reduction in protein carbonylation and lipid peroxidation, 2) confirmed a significant role of NAC in regulation of hematological response, 3) revealed the relationship between changes in plasma total antioxidant status and hematological parameters, 4) disqualified ALA as thiol compound which could affect EPO secretion.

Conflict of Interest

There is no conflict of interest.

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