N-Acetylcysteine Attenuates Oxidative Burst by Neutrophils in Response to Ergometer Rowing with no Effect on Pulmonary Gas Exchange

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This study evaluated whether the reduction of the neutrophil oxidative burst by *N*-acetylcysteine improves pulmonary gas exchange during a six minute maximal ergometer row. Healthy trained oarsmen were double-blinded randomized to either *N*-acetylcysteine (6 g daily for three days) or placebo groups. As determined by the relative changes of the zymosan-stimulated luminol-enhanced chemiluminescence response, *N*-acetylcysteine suppressed the exercise-induced enhanced neutrophil oxidative burst response to rowing (−7 ± 6% vs. 17 ± 8%; $P < 0.05$). This was the case although the concentration of neutrophils remained similarly elevated above the pre-exercise level in both trials (to 5.4 ± 0.5 vs. 5.9 ± 0.6 × 10^9 cells × l^{-1}, respectively, $P > 0.05$). In the placebo and *N*-acetylcysteine groups, pulmonary ventilation increased and the arterial CO_2 partial pressure decreased to the same extent during exercise. Also, at the end of exercise the arterial O_2 partial pressure (77 ± 1 vs. 78 ± 1 mmHg), haemoglobin O_2 saturation (92 ± 1% vs. 93 ± 1%) and O_2 uptake (5.0 ± 0.2 vs. 4.9 ± 0.2 l × min^{-1}) were not significantly affected by *N*-acetylcysteine. Equally, two hours after exercise, the pulmonary diffusion capacity was reduced by 7 ± 2% below the pre-exercise with no significant influence of *N*-acetylcysteine. We conclude that the neutrophil oxidative burst to exercise does not influence pulmonary gas exchange during and after maximal rowing.

Key words: Blood gas variables, exercise, glutathione, oxygen uptake, pulmonary diffusion capacity, rowing.

Introduction

During maximal rowing, arterial oxygenation is impaired [7,23,24] and two hours into the recovery pulmonary diffusion capacity (DL_{CO}) is reduced [7–9,22]. Both findings could relate to an impaired alveolar-capillary membrane function. Thus only about half of the post-exercise reduction of DL_{CO} is caused by a reduced pulmonary capillary blood volume [9]. The other part of the reduction in DL_{CO} is related to the membrane component [7].

The cellular immune system is affected by exercise [29]. This effect is pronounced especially during rowing [26,27] because there is a higher level of sympathetic activation than during other types of exercise [12]. Activation of immune cells is of interest because neutrophils may initiate lung damage [10] as activated neutrophils adhere to the endothelium [20] with subsequent increased pulmonary vascular permeability [40]. Although cardiac output increases during exercise, neutrophils may remain 60–100 times longer in pulmonary capillaries than the red blood cells [11]. Also in lung tissue several indices of oxidative stress increase in response to exercise [43] leading to the hypothesis that the oxidative burst of neutrophils could affect the alveolar-capillary membrane.

N-Acetylcysteine has antioxidant activity in blood [1,33] as in the lung [3]. *N*-Acetylcysteine enhances cysteine availability for intracellular glutathione synthesis [36] and reduces neutrophil function [16]. Also *N*-acetylcysteine reduces symptoms in lung patients [30], and prevents deterioration of pulmonary function [31] and enhances pulmonary O_2 uptake in the critically ill patient [37]. Huupponen et al. [15] reported that *N*-acetylcysteine suppresses the oxidative burst of neutropfhils during exercise in humans, but it is not known whether *N*-acetylcysteine affects the O_2 uptake ($\dot{V}O_2$) in healthy subjects or if *N*-acetylcysteine maintains this influence on the neutrophils after exercise.

To test whether the neutrophil oxidative burst affects pulmonary gas exchange, elite oarsmen were treated with *N*-acetylcysteine in a placebo-controlled study. If the neutrophil oxidative burst affects the alveolar-capillary membrane function in healthy humans, we expected an improved pulmonary gas exchange during rowing and also that the reduction in the post-exercise pulmonary diffusion capacity would be attenuated by *N*-acetylcysteine.

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Methods

Nineteen male oarsmen (age 27 ± 1 yr, body mass 82 ± 2 kg, height 189 ± 2 cm; mean with standard error of the mean) participated in the study after giving informed consent. The protocol was approved by the Ethics Committee of Copenhagen (KF 02 - 132/93, KF 02 - 181/97) and by the National Board of Health (5312 - 348 - 1993). The subjects were not using any medication. On the day of the experiment the subjects reported to the laboratory 12 hours after their last meal. Exercise was prohibited in the 24 hours prior to the experiment.

On 2 days separated by 3 wk the subjects were randomly allocated to either a *N*-acetylcysteine (each pill was 300 mg; ASTRA, Copenhagen, Denmark) or a placebo (ASTRA) experiment in a double-blind cross-over design. Three grams of the respective supplement were administered with the morning and evening meals for 3 days before the experiment. Additional supplementation was given also 2 hours before the exercise protocol began. Thus 20 pills were taken each day for a period of 3 days. This dose represented the highest tolerable dose necessary to maintain subject compliance despite the low bioavailability of *N*-acetylcysteine [13]. The kinetics of *N*-acetylcysteine is illustrated in that an oral dose of ^{35}S-acetylcysteine increases plasma radioactivity for 24 hr [32]. Also, repeated doses of *N*-acetylcysteine maintain the plasma concentration of *N*-acetylcysteine [42]. *N*-Acetylcysteine disappears from the circulation with a half-life of about 1 hr [4], with cysteine being the major metabolite [36].

Exercise was performed on a rowing ergometer (Concept II, type C; Dreisacker, Morrisville, VT, USA) interfaced with a computer (Concept II) which displayed the split-time for every '500 m'. All rowers warmed up at an individually determined pace for 10 min followed by a 2 min recovery period after which the subjects rowed 'all-out' for six minutes. The power reported represents the average for the 6 min exercise bout. The exercise protocol was designed to simulate a 2000 m on-water competition, as the subjects were familiar with this type of exercise. A catheter (1.7 mm; 16 gauge) was placed through an antecubital vein and advanced to the superior caval vein in fourteen subjects and a 1.0 mm catheter (19 gauge) was inserted in the radial artery of the non-dominant arm in the other subjects. Heart rate was monitored by shortrange telemetry (Sport Tester PE 3000; Polar Electro, Kempele, Finland).

Respiratory values were recorded breath by breath (MedGraphics 2001, St. Paul, MN, USA) in all the subjects. The subject breathed through a mouthpiece while he wore a noseclip. After five minutes of rest, ventilation ($\dot{V}_E$), $\dot{V}O_2$, respiratory rate, carbon dioxide output ($\dot{V}CO_2$), and the end tidal partial pressures for O_2 (PET_{O_2}) and carbon dioxide (PET_{CO_2}) were averaged for every 15 s. During all-out rowing, the peak $\dot{V}O_2$ reflects $\dot{V}O_2$max [5].

Arterial blood samples for the CO_2 partial pressure (Pa_{CO_2}), the O_2 partial pressure (Pa_{O_2}), pH, haemoglobin O_2 saturation (Sa_{O_2}), and the concentrations of haemoglobin and lactate were obtained anaerobically in a heparinised syringe (QS50, Radiometer, Copenhagen, Denmark). Collections were made at rest, immediately before termination of rowing, and exactly two hours into the recovery. Blood gases were analysed at 37 °C on an ABL4 apparatus (Radiometer). The concentration of lactate in whole blood and in plasma were determined using a YSI 2300 apparatus (Yellow Springs Instruments Co., Inc., Oh, USA). The subjects were offered non-caffeinated soft drinks and water ad libitum after exercise but eating and drinking were prohibited within 30 min of blood sampling and the subjects remained supine.

The DL_{CO} was measured by the single-breath technique [19] in eight subjects, as previously described before and two hours after exercise [7,8]. The measurements were made with the subjects in the seated position. The DL_{CO} is assessed by the use of an inspiration of a dilute mixture of CO in O_2 and He. The first 700 ml of the exhaled volume were discarded and the following 600 ml were sampled. Inspiratory and expiratory concentrations of CO were measured with an infrared analyser (MasterLab Jäger, Würzburg, Germany).

The concentration of neutrophils in peripheral venous blood was measured immediately prior to exercise, following 15 min of supine rest, during the end of exercise, and one and two hours after termination of rowing in fourteen subjects. Three millilitres of blood was drawn into sterile tubes treated with potassium-EDTA and the neutrophil concentration subsequently determined using a cell counter (Technicon, New York, NY, USA). In addition, twenty millilitres of blood were drawn into tubes treated with 100 ml of 20 IU heparin. Neutrophils were separated by a two-step Lymphoprep gradient centrifugation (Nycomed) and dextran sedimentation technique.

Stimulated neutrophils converts O_2 into superoxide, which is reduced further to hydrogen peroxide [18]. The release of myeloperoxidase by neutrophils uses hydrogen peroxide. Superoxide that has escaped from the phagosome is reduced to peroxide, which oxidises glutathione. The reactive O_2 species are unstable and they return to the ground state in a matter of seconds to minutes accompanied by the release of photons. This light reaction, or chemiluminescence, was measured in a luminol-enhanced assay for six subjects. The assay was performed in a total volume of 1 ml in an LKB 1250 luminometer [17]. A cell suspension of 100 µl (5×10^5 cells) was stimulated with opsonised zymosan (10 mg × ml^{-1}) added to 700 µl of 5×10^{-8} M luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). The peak chemiluminescence was noted and results reported as percent change from baseline.

Venous blood sampling for the determination of plasma cysteine and glutathione was performed before and at the end of rowing in six subjects. The concentrations of cysteine and the reduced form of glutathione were measured on a high-performance liquid chromatography gradient system with fluorescent detection [2]. Blood was obtained in ice-cooled tubes to which serine/borate (20 nM) was added. Immediately thereafter the samples were centrifuged and 100 µl of plasma was obtained for derivation with monobromobimane frozen for later analyses.

The results were evaluated by the Friedman analysis of variance. Pairwise differences were determined using the Wilcoxon signed rank sum test. Statistical significance was set at the 95% confidence limit ($P < 0.05$). All data are presented as means ± standard error of the mean.

Results

The concentration of neutrophils increased similarly in response to exercise in both the placebo and the *N*-acetylcysteine experiments (Fig. 1). During and after exercise the chemiluminescence response by neutrophils was above the resting level (38.9 ± 3.0 vs. 45.5 ± 4.3 and 41.3 ± 3.8mv, respectively, with the corresponding values for *N*-acetylcysteine being 42.1 ± 2.7 vs. 38.8 ± 3.4 and 34.6 ± 3.7mv) and this response was suppressed by *N*-acetylcysteine (Fig. 1). The plasma concentration of cysteine decreased during exercise in both trials, whereas that of glutathione remained at the baseline level (Table 1). *N*-Acetylcysteine increased the cysteine concentration both at rest and during exercise. *N*-Acetylcysteine exerted no significant influence on the concentration of glutathione.

Work capacity was not affected by *N*-acetylcysteine (361 ± 11 vs. 362 ± 10 W). During rowing $\dot{V}_E$ was elevated and PET_{CO_2} and Pa_{CO_2} decreased below the resting levels. PET_{O_2} increased while Pa_{O_2} and Sa_{O_2} were diminished (Table 1). The concentration of haemoglobin increased. With the administration of *N*-acetylcysteine there was no significant effect on $\dot{V}O_2$ or on other respiratory variables. Also the changes in lactate concentrations and pH were the same with both placebo and *N*-acetylcysteine supplementation.

Compared to resting levels, DL_{CO} was reduced two hours after exercise with no significant effect of *N*-acetylcysteine (41 ± 2 vs. 41 ± 3 ml × min^{-1} × mmHg^{-1} at rest and 38 ± 3 vs. 39 ± 3 ml × min^{-1} × mmHg^{-1} after exercise; placebo vs. *N*-acetylcysteine).

Discussion

N-Acetylcysteine abolished the increased chemiluminescense of neutrophils both during and after maximal ergometer rowing. However, this effect of *N*-acetylcysteine on the neutrophils did not significantly influence work capacity, pulmonary gas exchange, oxygen uptake or the post-exercise reduction in pulmonary diffusion capacity.

Neutrophils

The increased concentration of neutrophils and the priming of neutrophils in response to rowing correspond to the responses demonstrated during other types of exercise [28, 29]. The stimulus to prime neutrophils during exercise may be related to cellular mobilization [38], sympathetic activation [39] or to the intense O_2 transport in blood. However, during rowing the increased chemiluminescence response was not higher than that previously reported although the concentration of catecholamines is two to three fold higher during maximal rowing than during other types of maximal exercise [12]. Also the redistribution of blood volume is extreme as cardiac output becomes elevated to above 30 l × min^{-1} during a maximal ergometer row [24].

The present study supports the observations by Huupoonen et al. [15] that *N*-acetylcysteine abolishes the neutrophil oxidative burst. A new finding was that *N*-acetylcysteine maintains its influence on the neutrophils for two hours after exercise. The pathway of neutrophil reactive O_2 species metabolism might be forwarded from precursors to the stages of more reactive oxidant formation due to the facilitation of myeloperox-

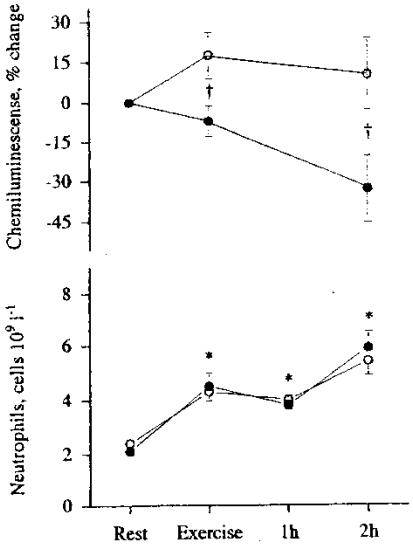

Fig. 1 Upper panel; the percent change of zymosan-stimulated luminol-dependent chemiluminescence response by neutrophilic granulocytes in response to maximal exercise either with placebo (open symbols) or *N*-acetylcysteine (closed symbols). Lower panel, the concentration of neutrophilic granulocytes before, during and after (one and two hours) maximal exercise with placebo or *N*-acetylcysteine. Values are mean with SEM. *= different from rest; †= different from placebo, $P < 0.05$.

Table 1 Variables with either placebo or *N*-acetylcysteine

	Placebo		*N*-acetylcysteine	
	Rest	Exercise	Rest	Exercise
$\dot{V}_E$ l × min^{-1}	11 ± 2	176 ± 5*	10 ± 2	168 ± 5*
fR breath × min^{-1}	14 ± 2	66 ± 2*	15 ± 2	64 ± 2*
PET_{CO_2} mmHg	42 ± 1	36 ± 1	41 ± 1	34 ± 1*
Pa_{CO_2} mmHg	39 ± 1	35 ± 2*	40 ± 1	35 ± 2*
$\dot{V}CO_2$ l × min^{-1}	0.3 ± 0.0	5.2 ± 0.2*	0.3 ± 0.0	5.1 ± 0.2*
PET_{O_2} mmHg	109 ± 2	120 ± 1	112 ± 3	120 ± 1*
Pa_{O_2} mmHg	102 ± 4	77 ± 4*	109 ± 4	78 ± 5*
pH	7.39 ± 0.01	7.04 ± 0.02*	7.37 ± 0.03	7.05 ± 0.01*
Lactate[1)] mmol × l^{-1}	1.7 ± 0.2	21.7 ± 1.3*	1.5 ± 0.1	21.4 ± 1.2*
Lactate‡ mmol × l^{-1}	0.6 ± 0.1	9.7 ± 0.6*	0.5 ± 0.1	9.5 ± 0.5*
Sa_{O_2}%	98 ± 0.4	92 ± 1.3*	98 ± 0.3	93 ± 0.9*
Haemoglobin mmol × l^{-1}	8.9 ± 0.1	10.2 ± 0.1*	8.9 ± 0.1	10.1 ± 0.1*
$\dot{V}O_2$ l × min^{-1}	0.3 ± 0.0	5.0 ± 0.2*	0.5 ± 0.1	4.9 ± 0.2*
fH bpm	63 ± 5	186 ± 2*	66 ± 6	187 ± 2*
Cysteine µmol × min^{-1}	25.0 ± 3.2	17.6 ± 1.8*	40.8 ± 4.4†	28.2 ± 6.0*†
Glutathione µmol × min^{-1}	2.6 ± 0.8	4.4 ± 1.7	3.2 ± 0.6	4.6 ± 0.9

Variables are the average and SEM. fH: heart rate, Pa_{O_2} and Pa_{CO_2}: partial pressure of oxygen and carbon dioxide in arterial blood; Sa_{O_2}: oxygen saturation of arterial haemoglobin; $\dot{V}_E$: pulmonary ventilation; $\dot{V}C_{O_2}$: expired carbon dioxide; $\dot{V}O_2$: pulmary O_2 uptake. The concentration of lactate are in plasma ([1)]) and in whole blood (‡). *different value compared to rest; † different value compared to placebo. $P < 0.05$

idase degranulation [18]. It is not clear how *N*-acetylcysteine achieves its effect. *N*-Acetylcysteine attenuates both chemotaxis and oxidative burst of the neutrophils, whereas their bactericidal activity is not affected [16]. *N*-Acetylcysteine may exert its action by a direct cellular effect. An attenuated chemiluminescence could also be related to increased concentration of glutathione within the neutrophils.

Intracellular glutathione has not been measured during exercise, but two studies have determined the extracellular glutathione in response to physical activity in humans [33,34]. Exposure of muscle cells to an oxidant challenge results in a rapid oxidation of intracellular glutathione, which is followed by a rapid efflux of oxidised glutathione [34]. This is substantiated by the finding of an unchanged concentration of blood glutathione (the reduced form), as also demonstrated by Sastre et al. [33]. Also, the blood content of total glutathione (reduced + oxidised) increases [34]. Although the liver may contribute to blood glutathione, hepatic blood flow is low during maximal rowing as indicated by virtually no clearance of indocyanine green injected into blood [24]. As cysteine is the precursor of glutathione [36], the *N*-acetylcysteine induced increased concentration of plasma cysteine is of importance for the intracellular synthesis of glutathione. Indeed haemolysis of red blood cells could contribute to a change in the concentration of glutathione in blood. With haemolysis of erythrocytes, plasma is coloured red, but we did not observe such a colouring during separation of the blood. Also the haematocrit increases in response to rowing [24] reflecting changes in plasma volume and cellular mobilisation [25].

Pulmonary O_2 transport

With pronounced acidosis during rowing, haemoglobin affinity for O_2 binding is reduced, and with a decrease in Pa_{O_2}, arterial O_2 desaturation is established [7,23,24] as confirmed in this study. The fact that arterial oxygenation is impaired during rowing suggests that a pulmonary O_2 diffusion limitation is of importance [24]. Exercise induces oxidative stress in the lung tissue [43], and we evaluated if activated neutrophils influence the alveolo-capillary membrane to an extent which could have an impact on pulmonary O_2 transport. Such speculation was based on the role of neutrophils in lung damage [10]. Also, activated neutrophils adhere to the endothelium [20] and immediately alter the pulmonary vascular permeability [40], which is related to neutrophil activation and reactive O_2 species [41]. However, Pa_{O_2} as Sa_{O_2} were reduced to the same extent in *N*-acetylcysteine and placebo experiments associated with a reduction in neutrophil oxidative burst. This finding indicates that activated neutrophils do not affect pulmonary O_2 transport to an extent which is reflected in blood gas analyses.

We also hypothesised that an effect of neutrophils and secondary of reactive O_2 species could maintain an influence on the alveolar-capillary membrane function after the ergometer row. This assumption was based on reports that DL_{CO} is reduced in the recovery from exercise [7–9,21,22,35]. Two hours after a maximal ergometer row DL_{CO} is reduced by ≈ 10% reflected in a reduction of both the pulmonary capillary blood volume and the membrane component of DL_{CO} [7]. Part of this reduced DL_{CO} is related to a re-distribution of blood away from the lungs towards the previously active muscles [9]. Why the membrane component of DL_{CO} is affected by exhaustive exercise is not known. It has been evaluated if an interstitial oedema is of importance for the membrane component of DL_{CO}, however, the results were negative [7].

An injury to the alveolo-capillary membrane is a likely explanation for the decrease in DL_{CO} (and its membrane component). In fact such an injury has been demonstrated in the horse [44]. Indirect evidence suggests that increased pulmonary vascular permeability also occurs in humans [14]. Thus, as a diuretic does not affect gas exchange during exercise or the post-exercise reduction in DL_{CO}, we took this finding to indicate that an interstitial pulmonary oedema [6] could arise on an inflammatory basis.

The present study did not include a direct evaluation of the alveolo-capillary membrane. However, about half of the reduction in DL_{CO} is related to the membrane component [9], whereby DL_{CO} would detect even a small change in membrane component. The finding that DL_{CO} was similarly reduced with placebo and *N*-acetylcysteine supplementation indicates that the activated neutrophils do not influence the alveolo-capillary membrane in response to rowing. Others than the neutrophils, e.g. the xanthine oxidase pathway, may generate reactive O_2 species, which could affect the alveolo-capillary-membrane function. However, the present results can neither support nor exclude such a hypothesis. We conclude that *N*-acetylcysteine does not improve the pulmonary gas exchange during rowing and that *N*-acetylcysteine does not enhance the pulmonary diffusion capacity two hours after maximal exercise.

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