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Protection against pulmonary O_2 toxicity by N-acetylcysteine

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Protection against pulmonary O_2 toxicity by N-acetylcysteine. P.D. Wagner, O. Mathieu-Costello, D.E. Bebout, A.T. Gray, P.D. Natterson, C. Glennow.
ABSTRACT: N-acetylcysteine (NAC) is a known antioxidant. We therefore investigated NAC as an agent protective against O_2 toxicity in the lung. Twelve dogs were anaesthetized with sodium pentobarbital and ventilated with 100% O_2 for 54 h. Five were given diluent and 7 intravenous NAC (loading dose prior to 100% O_2 ventilation of 150 $mg \cdot kg^{-1}$ and maintenance dose of 20 $mg \cdot kg^{-1} \cdot h^{-1}$). Every 6 h, physiological evaluation of the pulmonary circulation, mechanical properties, and gas exchange was performed. Post-mortem evaluation consisted of gross examination and weighing followed by light and electron microscopy. By both functional and structural criteria, NAC protected against the effects of 100% O_2. The NAC group developed significantly less increase in pulmonary vascular resistance, arterial carbon dioxide tension ($Paco_2$) and lung wet weight, while dynamic compliance was greater. NAC also delayed the development of abnormal ventilation-perfusion relationships and was associated with reduced pulmonary white cell accumulation, with less evidence of alveolar and interstitial oedema. NAC may well be worthy of evaluation as a therapeutic agent in human diseases characterized by oxidant damage.
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Interest in O_2 radical-mediated oxidant damage of various tissues has escalated in recent years with many diverse studies and the publication of several reviews [1–3]. Currently, much interest is focused on the cellular and molecular mechanisms of this damage, and there is a strong feeling that it may well be the basis of much morbidity and mortality. This seems to pervade multiple organ systems. For example, in the lung in ARDS (adult respiratory distress syndrome) the combination of white cell accumulation with the subsequent release of toxic O_2 radicals and the frequent need for high inspired O_2 concentrations (Po_2) form a mutually interactive "feed-forward" mechanism of parenchymal damage: each source may cause damage to lung cells, thereby increasing the accumulation of more white cells and the need for a still higher inspired Po_2 [4]. In the myocardium a component of the response to ischaemia is white cell accumulation (in the ischaemic area) with hypothesized cell damage from O_2 radicals released by these white cells [5].

There is therefore a clear-cut rationale for a search for antioxidant agents of potential human therapeutic benefit, even while basic research on mechanisms of oxidant damage continues. This is particularly urgent in the context of ARDS where mortality has been essentially unaltered at about 50% for many years despite a vast amount of basic and clinical research [6].

Many potentially useful agents have been examined, but most, even though effective in animal models, appear to be currently unsuitable for human administration. Thus, endotoxin and enzymes such as superoxide dismutase and catalase clearly afford protection but cannot yet be given to patients [7, 8]. Vitamin E has been used [9] but seems to work only in vitamin E deficient subjects [2]. A fascinating new possibility, fructose diphosphate [10], is currently under evaluation.

N-Acetylcysteine (NAC) is another potential agent for protecting against oxidant damage. Several reports document its antioxidant properties [11–15], although it is not clear whether these accrue: as a result of direct O_2 toxic radical transfer [16]; because NAC acts as a substrate for the natural antioxidant, glutathione [17]; because of direct reduction (compare reduction of H_2O_2 by glutathione [2]); for combinations of these reasons; or by means of some other mechanism as yet unknown. In the treatment of acetominophen poisoning it is felt

that it acts by promoting glutathione synthesis [17].

NAC has received attention as a mucolytic, both by inhalation [18] and more recently by oral administration [19, 20] and as an antioxidant for components of cigarette smoke [21, 22]. More recently, BERNARD *et al.* [23] demonstrated attenuation of physiological responses to endotoxin shock in sheep by NAC and PATTERSON *et al.* [15] showed considerably enhanced survival of rats exposed to 99% O_2 for 8 days (80% *vs* 30% for controls).

Because of the suitability for human use, the low incidence of side effects, the known antioxidant properties and the existing evidence of protection against O_2 toxicity and endotoxaemia, we felt further evaluation of NAC was highly desirable.

Our intention was to determine whether NAC, in the dosage used orally in humans for acetominophen poisoning (similar to that used intravenously by BERNARD *et al.* [23]), is of benefit in O_2 toxicity. No attempt to evaluate its protective mechanism has been made in this study, such questions being considered premature. Moreover, no dose-response relationship for NAC has been determined, again because we need to first ask whether NAC has any effect at all at the dose used in man. Our criteria for a protective effect are based on a combination of physiological and structural variables, and through both, we found substantial protection afforded by the drug.

Methods

Anaesthesia

General anaesthesia (in place of awake exposure to O_2 attempted for 2–3 days) was used for several reasons. Firstly, the nature of the pulmonary inflammatory response to long-term 100% O_2 is such that we felt it unethical to expose awake dogs to O_2. Secondly measurements made every 6 h for 2–3 days in which arterial and pulmonary arterial blood is taken and pressures recorded are very difficult to accomplish in the awake animal and pose additional welfare concerns. The choice of anaesthetic was sodium pentobarbital, 30 $mg \cdot kg^{-1}$ *i.v.* initially and then 25–100 mg bolus injections as necessary thereafter. While no anaesthetic is without disadvantages over such a long period of time, consultation with animal care veterinary staff produced no better agent than pentobarbital. Given judiciously, we found systemic cardiovascular instability was rarely a problem, and then only in the last 12–24 h of each study.

Muscle relaxation was achieved with *i.v.* administration of Pavulon 1–2 mg as needed, but always given together with pentobarbital.

Dose of O_2 and N-acetylcysteine

Duration of exposure was determined from preliminary experiments in four dogs which were followed (under anaesthesia) until death. In each, the clinical course was characterized by essential stability of cardiopulmonary indices for about 60 h followed by sudden, rapid deterioration with systemic hypotension, hypoxaemia and gross pulmonary oedema developing over about 2–6 h and resulting in death. Accordingly, we terminated all subsequent experiments electively after 54 h, allowing 10 sequential 6 hourly measurements over this time period.

Dosage of NAC followed published regimes. When used as an antidote for acetominophen overdose, the manufacturer suggests a loading dose of 140 $mg \cdot kg^{-1}$ and maintenance doses of 70 $mg \cdot kg^{-1}$ every 4 h [24]. BERNARD *et al.* [23] used 150 $mg \cdot kg^{-1}$ and 20 $mg \cdot kg^{-1} \cdot h^{-1}$ in their study of the effects of NAC in endotoxaemia in sheep, and we followed Bernard's regime exactly, also using the intravenous route. The loading dose was given 30 min prior to the start of 100% O_2 breathing and the maintenance dose was given by continuous infusion, by adding the necessary amounts of NAC to the maintenance 5% dextrose solution. NAC was given until death.

Physiological measurements made each 6 h

O_2 toxicity includes inflammation and oedema formation and these pathological changes can produce alterations in pulmonary mechanical properties, pulmonary blood pressure and flow relationships, and pulmonary gas exchange. Physiological variables concerned with each of these three broad areas were measured.

Mechanical properties were evaluated by: 1) conventional helium dilution measurement of functional residual capacity (FRC) in duplicate; and 2) measurement of dynamic compliance at maintenance breathing frequency (mean $14 \cdot min^{-1}$) as the ratio of tidal volume to the difference between end-inspiratory and end-expiratory tracheal pressures.

The pulmonary circulation was evaluated by conventional measurement of: 1) mean pulmonary artery pressure; 2) mean pulmonary artery wedge pressure; and 3) cardiac output. For pressure, P23ID Statham (Statham, Oxnard, CA) transducers were used, zero referenced to atrial level with the dogs supine and calibrated immediately prior to recording pressures every 6 h. Cardiac output was determined by the Fick principle from the multiple inert gas elimination technique mentioned below.

Gas exchange was evaluated by measurement of: 1) mixed venous and arterial Po_2, carbon dioxide tension (Pco_2), pH and O_2 saturation; 2) mixed expired O_2 and CO_2 concentrations; and 3) the multiple inert gas elimination technique for estimating ($\dot{V}A/\dot{Q}$) inequality. We used the IL 813 blood:gas electrode system calibrated prior to each sample and the IL 282 co-oximeter for saturation measurement. Arterial blood samples were analysed on the IL 813 within 30 s of being collected, and mixed venous samples immediately after the arterial measurement had been completed. For the inert gas technique we used our conventional approach [25, 26]

and a Hewlett-Packard 5890A gas chromatograph. Mixed expired O_2 and CO_2 levels were measured by mass spectrometry (MGA 1100, Perkin-Elmer, Pomona, CA) with calibration immediately prior to each set of measurements every 6 h. Mixing of expired gas was accomplished continuously by means of the same flow-through heated metal box used for collecting expired gas for the inert gas technique [27]. Oesophageal temperature was recorded at the time of blood sampling (Yellow Springs thermometer, Yellow Springs, Ohio).

Post-mortem histological evaluation

Immediately prior to sacrifice at 54 h, each animal was heparinized (10,000 U heparin *i.v.*) and then sacrificed by pentobarbital overdose. The lungs were removed intact from the chest and weighed. After inflation to 20 cmH_2O and examination of the pleural surfaces, heparinized, normal saline was pumped through the cannulated pulmonary artery until the pulmonary venous effluent was no longer visibly coloured. This was followed by perfusion with 2% buffered glutaraldehyde at the same pressure (15 mmHg above airway pressure) for approximately 15 min. The lungs were then immersed in 2% glutaraldehyde for 5 days, after which blocks were cut from the lower lobes (right and left lower, and cardiac). The upper lobes were generally not used because the initial glutaraldehyde perfusion only poorly fixed these lobes due to gravitational effects. As a result, they could not be kept sufficiently inflated during subsequent processing. The blocks were taken from both macroscopically normal and macroscopically abnormal regions in each dog. Light microscopy was subsequently performed after preparation of 5 μm sections with a Giemsa stain to enhance recognition of cell nuclei. Electron microscopy was also performed to examine cellular integrity and to measure the thickness of the blood:gas barrier.

Several variables were quantified to permit a statistical comparison of NAC treated and untreated dogs. By light microscopy, we counted the number of: 1) polymorphonuclear white cells (PMNs) per length of alveolar septum; 2) mononuclear cells (MNs) per length of alveolar septum; 3) PMNs and MNs free in the alveolar spaces; and 4) airways and blood vessels surrounded by oedema. Cell counts were determined by light microscopy at a magnification of 400 . Ten fields, chosen by systematic random sampling, were analysed in each of 33 slides, 20 of which were from four control animals, and 13 from three NAC treated animals. Numbers of cell sections and alveolar boundary length per unit area of lung parenchyma were estimated by standard stereological techniques [28] using an eyepiece graticule (10 10 square grid; distance between 2 test points, 0.02525 mm). We used the ratio between the above two parameters and expressed each cell count (PMNs and mononuclear white cells in alveolar wall and/or free in alveolar space) per unit length of alveolar boundary. By doing so, we took into account possible differences in the degree of expansion or collapse of the lung tissue between samples and/or animals.

By electron microscopy we determined the mean thickness of the thin part of the pulmonary blood-gas barrier.

Clinical management

Ensuring similar clinical management of all dogs was of prime importance. Measurements were made in the supine position (every 6 h), but inbetween, each animal spent 2 h in each of the lateral decubitus positions. The supine position was resumed for the 2 h preceding each set of 6 hourly measurements. Body temperature was kept >37.5°C by means of a heating blanket and cooling by ice was used if temperature exceeded 39°C. Intramuscular ampicillin (5 $mg \cdot kg^{-1}$ every 6 h) was given prophylactically throughout the study. All dogs were sighed to 20 cmH_2O hourly and kept on 5 cmH_2O positive end-expiratory pressure throughout. Fluid maintenance was by 5% dextrose for caloric support, 1 $l \cdot day^{-1}$ per 12.5 kg body weight, and fluid output was measured by urinary catheter. We used normal saline to flush catheters and for infusion of the inert gas solution used in gas exchange measurements. Acid-base status was monitored by measurement of Po_2, Pco_2 and pH every 2 h throughout the entire study, and sufficient $NaHCO_3$ was given to keep arterial pH at, or above 7.35. All animals were purposefully hyperventilated to an initial arterial Pco_2 of 30 torr because it became evident that arterial Pco_2 tended to rise several torr by the end of the study. The low initial Pco_2 obviated the need for subsequently altering the ventilation settings.

Pulmonary artery wedge pressure was measured regularly and used to dictate minor fluid administration adjustment. Because wedge pressure remained remarkably constant throughout the study, this adjustment was rarely required. Finally, suction of large airways was performed whenever audible respiratory sounds or visible secretions became evident, but this was also seldom needed.

Protocol

A total of 16 conditioned mongrel dogs were studied, 4 of which were room air-ventilated control animals not given NAC. Five received vehicle and the other 7 NAC as described above. Of these 12, a total of 4 (1 placebo and 3 NAC treated) constituted the initial group of dogs that were followed until death from fulminant pulmonary oedema and hypotension. Physiological data for the first 54 h of these dogs are used in the ensuing analysis, but histological and morphometric data were not used. Physiological data from the other 8 dogs sacrificed at 54 h were obtained and used; morphological results for all except one NAC treated dog (which suddenly died between 48 and 54 h) are similarly included.

In summary, all 12 O_2 ventilated dogs and the four

air controls contributed physiological data through 54 h except for the above mentioned NAC treated animal which could be used only to 48 h; 4 untreated and 3 NAC treated dogs provided morphological data, as did the four controls. The criterion for acceptance of a dog for either physiological or morphological study was a normal arterial Po_2 (>550 torr) at the time of measurement or sacrifice, respectively.

Animals were prepared with two peripheral *i.v.* lines, a pulmonary arterial balloon-tipped catheter and a systemic arterial line. After initial measurements were made breathing room air, the experimental animals were switched to 100% O_2 and measurements made 30 min later. These were repeated every 6 h for a total of 10 sets collected over 54 consecutive hours of 100% O_2 breathing.

Each set of 6 hourly measurements consisted of the following steps, in order: 1) calibration of mass spectrometer and pressure transducers; 2) measurement of mixed expired oxygen fraction (Fo_2) and carbon dioxide fraction (Fco_2); 3) measurement of systemic and pulmonary arterial phasic and mean pressures and tracheal pressure; 4) collection of 7 ml duplicate blood and mixed expired gas samples for measurement of $\dot{V}A/\dot{Q}$ inequality by the multiple inert gas elimination technique; 5) collection of 2 ml duplicate blood and samples for measurement of Po_2, Pco_2, pH and O_2 saturation; 6) recording of minute ventilation (by a calibrated Wright respirometer), respiratory frequency and oesophageal temperature; and 7) duplicate measurements of FRC by helium dilution using 10% helium in air and the mass spectrometer. Rebreathing of helium was accomplished by means of a 1 litre syringe connected directly to the endotracheal tube.

Statistical analysis of physiological data

For each variable examined, two questions were posed: 1) was there a difference between NAC treated and untreated animals in the time of initial development of abnormality; and 2) averaged over hours 42–54, was there a difference in the magnitude of the abnormality between treated and untreated animals? For each variable the first was answered by: a) calculating separately for each dog the mean of the first 3 sets of values (hours 0, 6 and 12); b) normalizing all data (of each animal) to this mean to account for initial inter-animal differences; c) finding for all 12 dogs combined, the 95% confidence limits of the first 12 h values about the normalized mean; d) locating the time of the first measurement that exceeded the 95% confi-

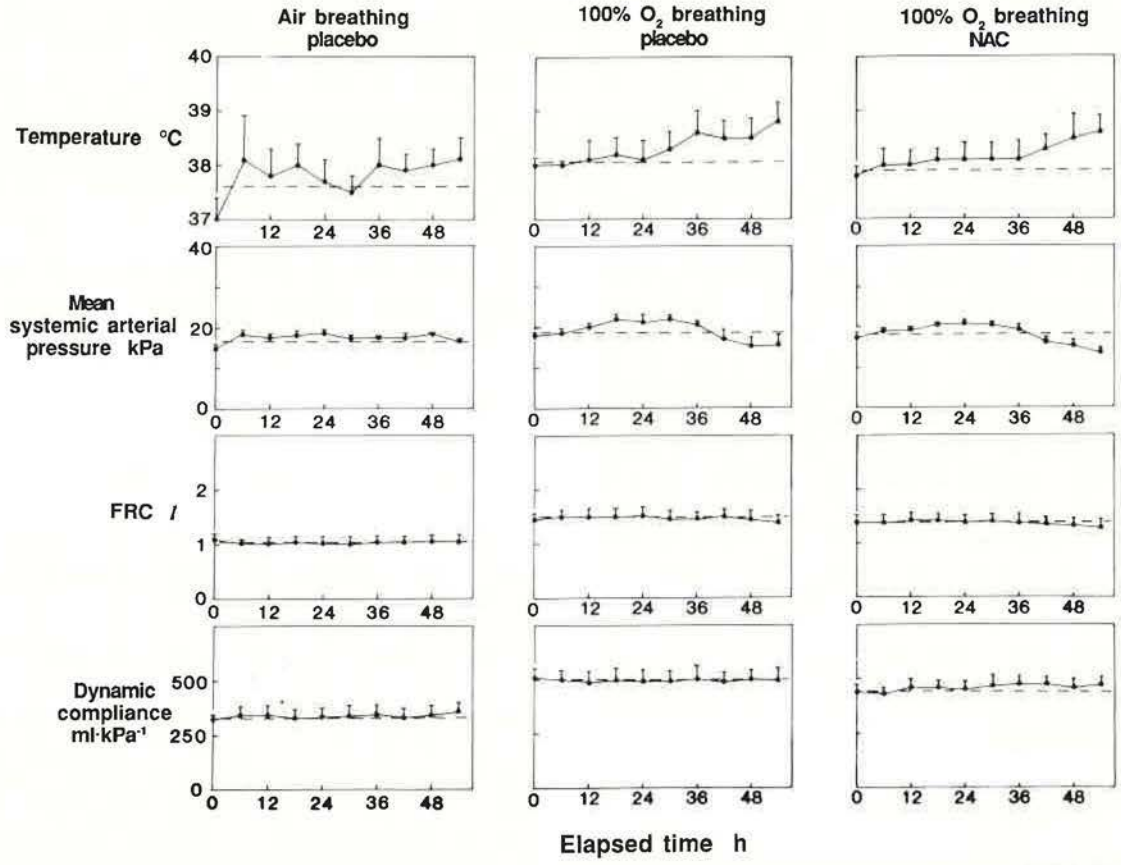

Fig. 1. – Time course of temperature, arterial blood pressure, functional residual capacity (FRC), and dynamic compliance over 54 h of 100% oxygen breathing (mean±SEM). Lefthand panels refer to control air breathing animals, middle to control O_2 breathing animals, and righthand panels to those given N-acetylcysteine (NAC). Changes from baseline are small for these variables, and only dynamic compliance was significantly different between control and treated groups at the end of the study. A rise in body temperature and fall in systemic arterial pressure occurred over the last third to one-half of the study.

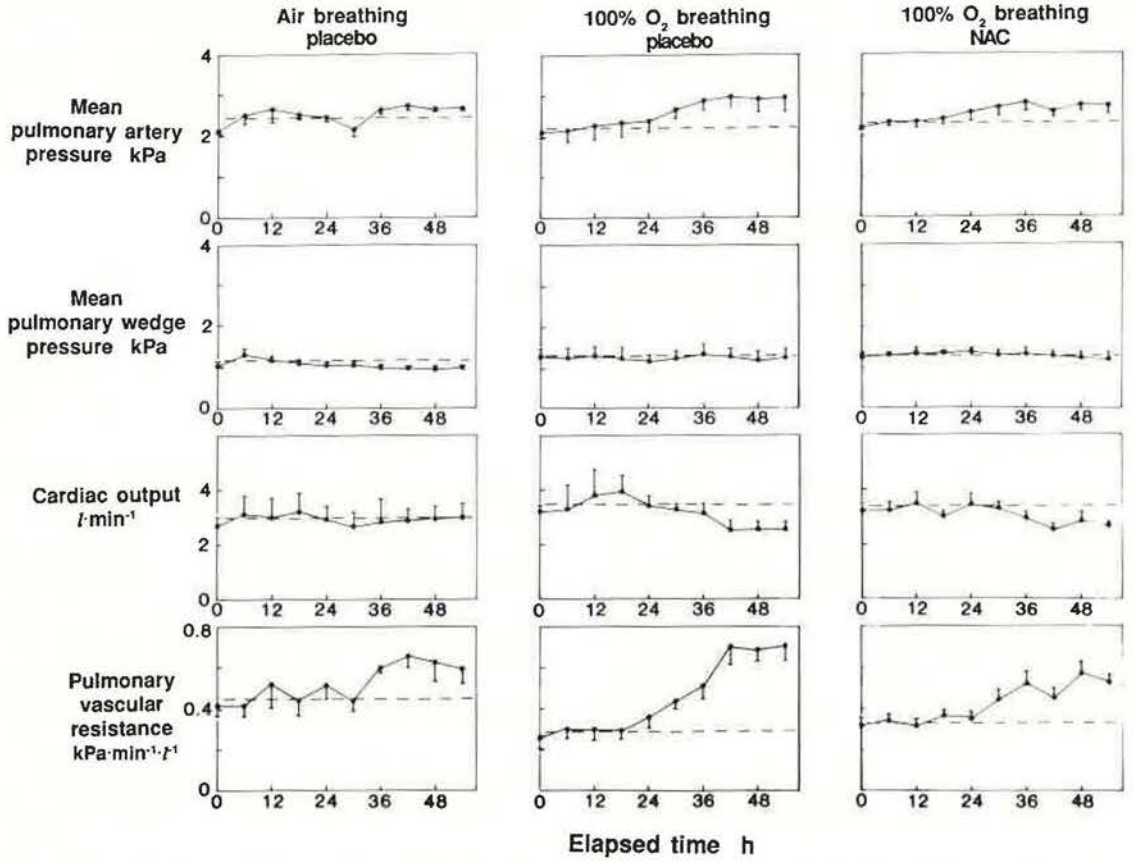

Fig. 2. – Time course of pulmonary artery pressure, pulmonary artery wedge pressure, cardiac output and pulmonary vascular resistance over 54 h of breathing 100% oxygen (mean±SEM). Animal groups are as in figure 1. Notice a larger rise in pulmonary vascular resistance in the O_2 breathing control animals which is shown to be the result of a greater increase in pulmonary artery pressure. Wedge pressure and cardiac output, on the other hand, behave similarly in the three groups. By the end of the study, pulmonary vascular resistance was 260% of initial values in the O_2 breathing control group, but only 170% of baseline in the N-acetylcysteine (NAC) treated group.

dence limit for each animal; and e) comparing those times by an unpaired t-test between the 5 control and 7 NAC treated animals. For a variable that always stayed within the 95% confidence limit, a time of 60 h was assigned (the last data being accumulated at 54 h) as the most conservative approach. It is only possible that this is an underestimate of the time at which variables first became different from baseline. Because the data were collected every 6 h only, times for each dog can be determined with only this degree of time resolution: the average over all dogs in each group need not have been a multiple of six, however.

To answer the second question, steps a) and b) were performed as above and then averages of the 3 normalized data sets for hours 42, 48 and 54 were compared by an unpaired t-test between the 7 NAC treated and 5 untreated animals.

Analysis of morphometric data

Unpaired t-tests were used to compare the variables described above obtained from the 4 untreated and 3 NAC treated animals whose lungs were subjected to morphometric analysis.

A similar analysis was performed for the lung wet weights, body weights and ratios of lung to body weight and lung weight to FRC.

Results

Time course of physiological variables

Figures 1, 2 and 3 display the time course of the twelve key variables that were measured. In each figure the lefthand panels reflect the 4 air breathing controls, the middle panels reflect the 5 untreated O_2 ventilated animals, and the righthand panels the 7 dogs given NAC and 100% O_2. The air breathing control dogs essentially showed no deleterious effects of 54 h of anaesthesia. Thus, temperature stabilized at 38°C after the initial measurement, as did arterial blood pressure, lung volume and dynamic compliance (fig. 1). Arterial Po_2 certainly did not fall nor did Pco_2 rise over the course of the study, and arterial pH also remained constant, without the need for bicarbonate infusion. Ventilation-perfusion inequality certainly did not increase, and if anything, tended to improve (fig. 3,

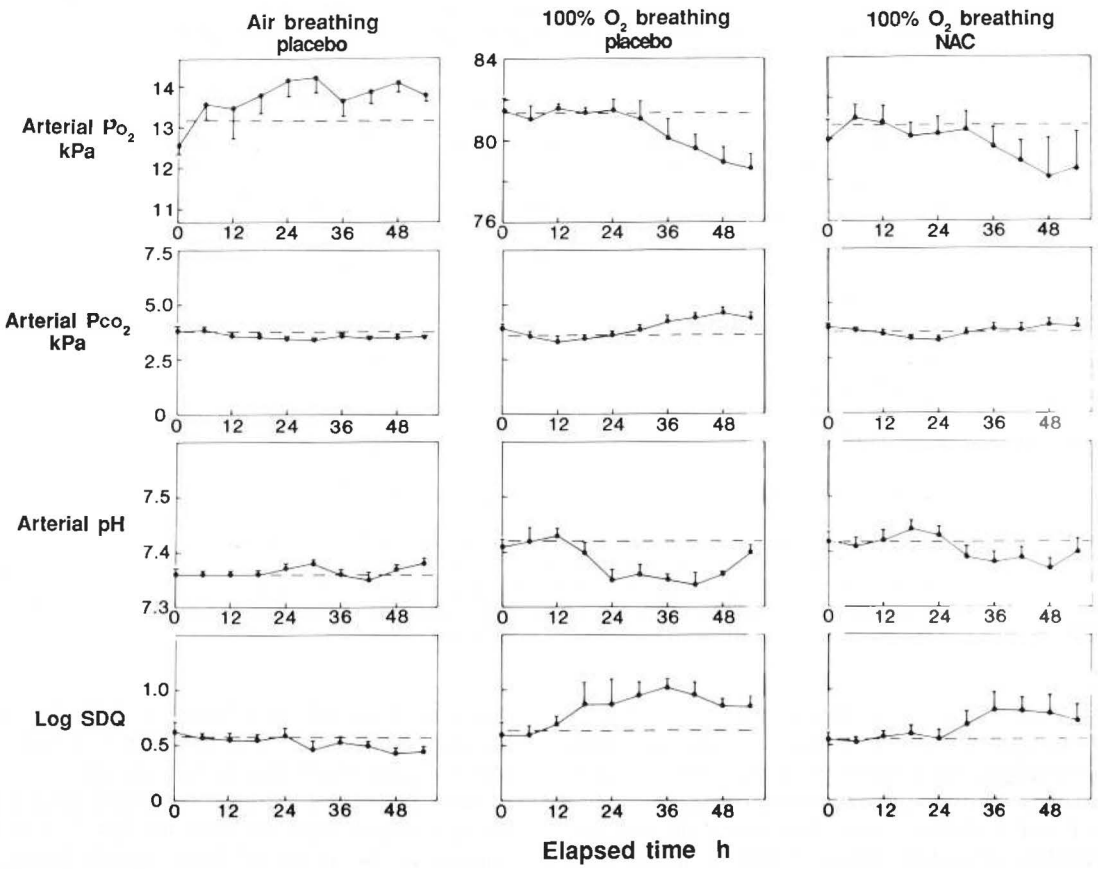

Fig. 3. – Time course of arterial oxygen tension (Po_2), carbon dioxide tension (Pco_2) and pH and dispersion of the second moment of the ventilation-perfusion (V_A/Q) distribution on a log scale (log SDQ) over 54 h of oxygen breathing (mean±SEM). Both pH and Pco_2 deviated from initial conditions earlier, and to a greater extent in the control animals, but Po_2 was minimally changed in all. Ventilation-perfusion inequality developed much earlier in the O_2 breathing control group than in the N-acetylcysteine (NAC) treated group, and none developed in the air breathing controls.

second moment of perfusion distribution on a log scale log SDQ). While pulmonary artery and wedge pressures and cardiac output each remained stable, there was a small increase in pulmonary vascular resistance (of 38%) first seen at 36 h and maintained thereafter. In the O_2 ventilated dogs, the variables used in the clinical management, which were designed to be held to normal values, changed little (temperature, pulmonary wedge pressure, arterial Po_2 and arterial pH). Other variables changed considerably, notably pulmonary vascular resistance and log standard deviation (second moment on a log scale) of the perfusion distribution. These three figures also show that some variables, particularly log standard deviation and blood H^+ concentration, appear to be increased earlier after O_2 breathing starts in untreated animals than in NAC treated

Table 1. – Time at which changes are first observed in O_2 ventilated dogs, (hours, mean±SEM)*

Variable	Untreated	NAC Treated	p**
Log SDQ	18.0±4.7	41.1±3.3	0.01
Arterial $[H^+]$	22.8±1.2	41.1±4.2	0.003
Mixed venous $[H^+]$	25.2±1.2	41.1±5.3	0.02
Mixed venous Pco_2	33.6±2.4	47.1±5.0	0.03
Arterial Pco_2	39.6±2.4	51.4±4.5	0.03
Mixed venous Po_2	44.4±5.9	57.4±2.6***	0.02
$\dot{V}co_2$	46.8±5.5	57.4±2.6***	0.04
$\dot{V}o_2$	25.2±4.4	41.1±7.2	0.06

*: Criterion is time at which a variable first differs from baseline by more than 2 SD of the values obtained during the first 12 h; **: probability that untreated and treated dogs did not differ; ***: a value of >54 h is possible because if no change in a variable occurred over the entire 54 h an appearance time of 60 h was used (as the most conservative estimate). Log SDQ: second moment of perfusion distribution on a log scale; $[H^+]$: hydrogen ion concentration; Pco_2: carbon dioxide tension; Po_2: oxygen tension; $\dot{V}co_2$: rate of CO_2 production; $\dot{V}o_2$: rate of O_2 uptake; NAC: N-acetylcysteine.

Table 2. – Physiological changes over time in O_2 ventilated dogs (mean values during hours 42–54 expressed as % of mean values during hours 0–12±SEM)

Variable	Untreated	NAC Treated	p*
Pulmonary vascular resistance	260.4±23.4	168.7±13.1	0.0004
Mean pulmonary artery pressure	139.7±7.8	114.5±3.9	0.002
Arterial P_{CO_2}	124.5±2.2	106.6±4.1	0.001
Mixed venous P_{CO_2}	129.1±2.3	111.5±4.7	0.003
Mixed venous P_{O_2}	77.7±3.1	102.6±6.2	0.002
Dynamic compliance	99.4±2.0	106.0±1.5	0.005
$\dot{V}_{O_2}$	132.3±3.3	118.2±2.0	0.0002
$\dot{V}_{CO_2}$	115.8±4.3	109.2±1.9	0.06
Arterial P_{O_2}	97.4±0.5	97.2±1.1	NS
Log SDQ	161.6±16.4	144.1±11.1	NS
Mean systemic arterial pressure	85.2±6.8	80.8±3.9	NS
Cardiac output	77.2±4.6	83.0±4.2	NS
Mean pulmonary wedge pressure	97.3±6.6	94.8±6.4	NS
Oesophageal temperature	101.3±0.3	101.3±0.4	NS
FRC	97.3±1.4	96.4±1.5	NS
Arterial $[H^+]$	114.1±3.7	108.8±3.4	NS
Mixed venous $[H^+]$	117.7±4.2	110.4±3.1	NS

*: probability that untreated and treated dogs did not differ; NS: not significant; P_{CO_2}, P_{O_2}: carbon dioxide, oxygen tension, respectively; $\dot{V}_{O_2}$: rate of O_2 consumption; $\dot{V}_{CO_2}$: rate of CO_2 production; Log SDQ: second moment of perfusion distribution on a log scale; FRC: functional residual capacity; $[H^+]$: concentration of hydrogen ions; NAC: N-acetylcysteine.

dogs. For other variables the total change over 54 h of O_2 breathing appears to be greater for the untreated animals, especially for pulmonary artery pressure, pulmonary vascular resistance and arterial P_{CO_2}.

Tables 1 and 2 analyse these data using the statistical approaches described above. Table 1 shows that increased ventilation-perfusion inequality is the first abnormality to be detected in untreated O_2 breathing animals (18 h). This is followed about 6 h later by increases in arterial (and mixed venous) H^+ concentration without altered blood P_{CO_2}, indicating a metabolic acidosis. Twelve hours later (at about 36 h) arterial and mixed venous P_{CO_2} both rise, presumably mostly due to developing $\dot{V}_A/\dot{Q}$ mismatch, and this is soon followed by an increase in metabolic rate. All of these abnormalities are delayed in their onset in the NAC treated dogs.

In the meantime, pulmonary vascular resistance begins to rise, arterial P_{O_2} falls minimally, body temperature rises and cardiac output falls slightly (all at 30 h in both O_2 breathing groups). At 36–42 h mean systemic blood pressure begins to fall. Indices reflecting mechanical lung properties (FRC, dynamic compliance) change minimally in both groups.

Table 2 gives for both O_2 ventilated groups, the mean values of many variables over the last 12 h of the study relative to the mean of those values during the first 12 h. The order of appearance in this table reflects the magnitude of the difference between NAC treated and untreated animals and the associated statistical significance of such differences. It is evident that the haemodynamic indices of pulmonary vascular obstruction are most affected followed by gas exchange variables and metabolic rate.

Post-mortem lung weights

Table 3 expresses lung wet weight and its ratios to body weight and to initial and final FRC values. Wet to dry weight ratios could not be measured because the lungs were needed for morphometric analysis and could

Table 3. – Post-mortem gravimetric data (mean±SD)

Variable	Units	F_{IO_2}: NAC:	0.21 No	1.0 No	1.0 Yes	p for NAC effect
Lung wet weight	$g \cdot kg^{-1}$		248±48	469±16	382±71	0.03
Body weight	kg		24.1±3.3	30.8±3.1	29.1±7.7	NS
Lung wt/body wt ratio	$g \cdot kg^{-1}$		10.3±1.0	15.4±2.0	13.3±1.1	NS(0.08)
Initial FRC	l BTPS		1.08±0.31	1.49±0.26	1.40±0.31	NS
FRC, hour 54	l BTPS		1.05±0.28	1.41±0.28	1.31±0.39	NS
Lung wet wt/initial FRC	$gm \cdot l^{-1}$		243±64	310±59	236±17	0.03
Lung wet wt/final FRC	$gm \cdot l^{-1}$		246±60	334±66	240±3	0.04

p: probability that data from treated and control O_2 ventilated animals did not differ; NS: not significant; F_{IO_2}: fraction inspired oxygen; NAC: N-acetylcysteine; FRC: functional residual capacity.

not be dried. Since it was necessary to use mongrel dogs of many breeds, wet/body weight ratios tended to be noisy and it is felt that normalizing to FRC may be a more appropriate tactic to account for extraneous differences in wet weight. Even so, air ventilated controls had significantly lower wet/body weight ratios than either O_2 ventilated group ($p<0.01$), and the untreated O_2 ventilated group tended ($p=0.08$) to have higher wet/body weight ratios than NAC treated animals. As shown, the untreated dogs had 23% heavier lungs despite similar body weights and higher ratios of wet weight to either initial or final (54 h) FRC. The wet weight to final FRC ratios are, in fact, 39% greater for the untreated O_2 ventilated dogs than for those given NAC.

We interpret the composite data of table 3 to indicate a moderately more oedematous lung in the untreated animals than following NAC therapy, and also that O_2 ventilation increased wet/body weight ratios compared to air breathing control conditions.

actually had more ($p<0.05$) septal polymorphs than the NAC treated dogs, but far fewer vessels with perivascular cuffing commensurate with the gravimetric data. Electron microscopic data from macroscopically normal areas revealed no differences in blood:gas barrier thickness (0.65 μm mean) amongst the three groups.

Discussion

Summary of results

Three lines of evidence point to a protective role of NAC in O_2 toxicity in the current experimental setting: physiological, gravimetric and morphological. That these are technically independent observations strengthen this conclusion. The untreated O_2 ventilated lungs either became abnormal sooner or became more abnormal (or both) than the NAC treated lungs.

The nature of these differences is likely to be of clinical significance: less inflammatory reaction, less oedema formation, less change in haemodynamic, mechanical and gas exchange properties of the lungs are all likely to result in reduced morbidity if the same effects occur in patients given NAC.

Table 4. – Morphometric variables

Variable (cells per mm of alveolar septum)	F_{IO_2}: / NAC:	0.21 No n=4 mean±SD	1.0 No n=4 mean±SD	1.0 Yes n=3 mean±SD	p*	p**	p***
Septal polymorphs		5.6±1.5	9.9±5.8	2.7±0.8		<0.05	<0.05
Septal mononuclear cells		9.7±1.1	35.1±22.6	18.7±5.6		<0.05	
Total septal white cells		15.3±1.9	45.1±27.3	21.4±5.7			
Alveolar polymorphs		0.2±0.1	0.8±1.1	0.2±0.2			
Alveolar mononuclear cells		1.7±0.8	2.1±1.5	0.6±0.4			
Total alveolar white cells		1.9±0.9	2.9±2.5	0.8±0.4			
Total white cells, alveolar plus septal		17.2±2.3	47.9±28.8	22.1±5.6			
Number of vessels with perivascular oedema, per 100 vessels		11.5±3.7	52.5±21.5	19.3±11.8	<0.01		<0.05

*: probability that room air ventilated and untreated O_2 ventilated groups are not different; **: probability that room air ventilated and NAC treated O_2 ventilated groups are not different; ***: probability that NAC and untreated O_2 ventilated groups are not different; F_{IO_2}: fraction inspired oxygen; NAC: N-acetylcysteine.

Post-mortem appearance and morphometry

Visual inspection of the lung surfaces following removal revealed essentially no macroscopic atelectasis or haemorrhage of subpleural regions in both the NAC treated and air breathing control dogs. In contrast, the untreated O_2 breathing animals had many purplish areas on the lung surface, mostly in the lower lobes in the dependent (dorsal) areas. These areas did not clear with inflation and were not due simply to atelectasis.

Table 4 summarizes the light microscopic data from the three groups of dogs, using all animals whose arterial P_{O_2} values exceeded 550 torr at sacrifice. It shows that 100% O_2 had greater effects in the untreated animals, causing more polymorph accumulation and perivascular cuffing. There was also more evidence in the untreated animals of pulmonary arterial plugging with cellular aggregates. The air breathing control dogs

Prophylactic vs *therapeutic nature of the present study*

A specific design criterion of the present study was to give the loading dose of NAC 30 min prior to commencing breathing 100% O_2 and to continue NAC until sacrifice some 54 h later. On the hypothesis that, directly or indirectly, NAC produces its protective effect through interference with O_2 toxic radicals, the agent would probably be less effective if given only after O_2 had been breathed for some time. Therefore, until shown otherwise, NAC may not be as useful in

the treatment of established lung disease caused by O_2 breathing. Should NAC come to human clinical evaluation, it would certainly seem advisable to give the drug as soon as possible after a patient has been identified for inclusion in such a study.

Stage of development of O_2 toxicity in the present study

In this particular experiment, O_2 toxicity had not progressed to an advanced state. Thus, arterial Po_2 was still at normal levels (>550 torr) and FRC had essentially not changed at the time of sacrifice. While there is clear-cut evidence of both physiological and morphological abnormality by 54 h in this model, the conclusions are limited to this time frame.

Beyond 54 h the anaesthetized dog becomes too unstable and difficult to manage clinically to permit a longer term evaluation of NAC during O_2 breathing to be undertaken.

One might ask whether the pathological changes we observed were, in fact, caused by O_2 toxicity, or on the other hand, by nonspecific reactions to prolonged anaesthesia, artificial ventilation, and possibly superimposed infection. The most direct way to answer this question is to include a group of air breathing animals as a control series. These are shown in figs 1–3 and it seems clear-cut that aside from a modest increase in pulmonary vascular resistance late in the study (fig. 2), the air breathing controls had no worsening of lung function. It seems reasonable then to conclude that we were in fact observing effects of 100% O_2 and not just effects of time. Moreover, the nature of the changes we observed agree with those of CRAPO *et al.* [29] who demonstrated oedematous alveolar septa, and in particular, neutrophil influx. We did not observe macroscopically or microscopically systematic evidence of pneumonia (airway secretions, alveolar consolidation with bacteria-laden inflammatory cells) and it should be recalled that the animals were treated with antibiotics throughout. No focal pyogenic pulmonary lesions were detected either.

Amount of NAC used and its route of administration

It has been pointed out that the current study greatly taxes laboratory resources. As a result, we felt that dose-response analysis of NAC effects during O_2 breathing would be premature. A satisfactory initial goal was, therefore, to answer only the question of whether NAC at the dose used by others was actually protective of the lung by any criteria. The doses used (150 $mg \cdot kg^{-1}$ bolus and 20 $mg \cdot kg^{-1} \cdot h^{-1}$ maintenance *i.v.*) are essentially identical in quantity or method of administration to that recommended in acetominophen overdose [24] and in the study of BERNARD *et al.* [23] who found similar protective effects of NAC in conscious sheep infused with endotoxin. They were also similar, both in terms of loading and maintenance values, to the amount used by PATTERSON *et al.* [15] in conscious rats exposed to O_2 for several days.

The question of dose-response relationships will have to await further studies.

Work of other authors

As mentioned, BERNARD *et al.* [23] in 1984 and PATTERSON *et al.* [15] in 1985 used NAC in conscious sheep given endotoxin and in conscious rats given 99% O_2, respectively. Both groups used the same route, scheme and dose of NAC as in the present study, commencing with NAC at, or before, the experimental insult of O_2 or endotoxin. BERNARD *et al.* found that NAC attenuated haemodynamic and lung mechanical responses to endotoxin, and reduced the rise in pulmonary lymph flow caused by endotoxin. PATTERSON *et al.* found encouraging protective effects of NAC by different criteria: survival. After 7 days in 100% O_2 young rats (180–226 g) had a 78% mortality without NAC reduced to 28% with NAC. Older rats (240–269 g) had a mortality of 83% reduced to 43% with NAC. This study did not address gravimetric, physiological or morphological variables. LAMBERT and GALINSKY have also reported in an abstract [11], beneficial effects of NAC given intraperitoneally to rats subjected to hyperoxia.

Thus, all of these studies tend to agree with the present findings, and together form a considerable body of evidence crossing three animal species that NAC can partly protect the lung and possibly the entire organism against the deleterious effects of toxic O_2 radicals.

NAC has also been found to beneficially interfere with oxidants in cigarette smoke [21] and, in a case report, to be of benefit in a patient with pancreatitis [30].

Mechanism of NAC effect

This study was not designed to elucidate the mechanism by which NAC protects the lung, nor are we equipped to study this question at the biochemical level. However, it is known that NAC may serve as a glutathione precursor in the liver [17] and, in addition, NAC is itself considered to be a potent free-radical scavenger [1, 16]. It seems possible, therefore, that NAC could act by either, or even both of these pathways in protecting against O_2 radicals, depending on the access of NAC to the sites at which the O_2 species are being produced or transported. It might also directly reduce compounds such as H_2O_2.

Of particular interest is the potential interaction between 100% O_2 inhaled into alveolar spaces and the polymorphonuclear white blood cells (PMNs) drawn into the lung presumably by the pulmonary macrophage response to O_2 breathing [4]. Both inhaled O_2 and PMNs are well-known to give rise to toxic O_2 radicals which can injure the lung. Consequently, one can easily imagine an interactive "positive feedback" situation wherein the respired 100% O_2 causes tissue

damage that attracts PMNs that cause further damage, requiring higher O_2 concentrations clinically and increasing the chemotaxis of PMNs. If NAC could reduce the number of free O_2 radicals (whether produced directly from inhaled O_2 or from attracted PMNs), there would be a rational basis for its observed effect.

The present study demonstrates reduced numbers of PMNs together with less oedema in the NAC treated dogs. We cannot, however, conclude from this that NAC is protecting the lung by some direct action on white cell-derived radicals. Presumably, to the extent that the hyperoxic damage stems initially from radicals produced from inhaled O_2, one would expect less PMN accumulation and less oedema after NAC, whether or not NAC was also interfering with cell damage caused by radical release from PMNs.

The observation that NAC protects normoxic sheep given endotoxin [23] as well as hyperoxic dogs does suggest that, to the extent NAC is acting similarly in both studies, one effect is on the PMN, an element of cell damage.

Sequence of physiological changes in O_2 toxicity

The untreated dogs in the present study provide a chronicle of the sequence of physiological changes during O_2 breathing. As noted, we followed variables concerned with gas exchange, haemodynamics and lung mechanical properties every 6 h for 54 consecutive hours. The first index to show abnormality was that describing the amount of ventilation-perfusion mismatching which was abnormal by 18 h on average (table 1). It is of interest that BLOCK and FISHER [31] found evidence of pulmonary endothelial dysfunction in the rat at this time also. However, CRAPO *et al.* [29] could find no morphological changes in the rat up to 40 h. If these times apply to the anaesthetized dog, it suggests functional changes considerably precede structural effects.

The second variable to change was blood pH (both arterial and mixed venous). Acidosis developed by 24 h and without change in blood P_{CO_2}. In other words, a metabolic acidosis was detectable at 24 h. It was only of minor proportions (pH>7.35) but enough to reach significance (table 1) and imply early interference to cell function. The NAC treated animals, despite the same initial pH, did not develop metabolic acidosis nor $\dot{V}_A/Q$ inequality until 41 h of O_2 breathing.

A small rise in temperature and in the rate of O_2 update ($\dot{V}O_2$) was also seen about 24–30 h, and pulmonary vascular resistance began to rise at the same time (figs 1–3, table 1).

Only at 36 h did blood P_{CO_2} and P_{O_2} begin to deteriorate, and the changes were small. In particular, arterial P_{O_2} fell only by some 20 torr over the entire study from a mean of 610 torr to a mean of about 590 torr (fig. 4), a negligible amount for an animal breathing 100% O_2. Assuming it reflects the development of intrapulmonary shunting, the size of such a shunt is of the order of only 1% of the cardiac output. Presumably, if arterial P_{O_2} had been measured while breathing room air for short periods every 6 h (the animal otherwise on 100% O_2), we would have observed a much earlier and more substantial relative fall in arterial P_{O_2} reflecting the early development of $\dot{V}_A/Q$ mismatching.

Only in the last 6–12 h of the observation period of 54 h did systemic arterial pressure begin to drop and FRC start to decrease. The changes in both were small, however (fig. 1).

While changes in lung compliance could alter ventilation distribution and the increased pulmonary vascular resistance could be associated with altered bloodflow distribution, the development of considerable $\dot{V}_A/Q$ mismatching occurred earlier than measurable changes in compliance or haemodynamics. Thus, we do not have a clear-cut explanation for the pathophysiological basis of the altered $\dot{V}_A/Q$ relationships developing as early as they did.

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Protection contre la toxicité pulmonaire de l'oxygène par la N-acétylcystéine. P.D. Wagner, O. Mathieu-Costello, D.E. Bebout, A.T. Gray, P.D. Natterson, C. Glennow.

RÉSUMÉ: La N-acétylcystéine (NAC) est un anti-oxydant connu, qui a été utilisé chez l'homme pour les surdosages d'acétominophène. C'est la raison pour laquelle on a considéré qu'il était intéressant d'investiguer son action protectrice contre la toxicité de l'oxygène au niveau du poumon. Douze chiens ont été anesthésiés au pentobarbital sodique et ventilés de façon continue avec de l'oxygène à 100% pendant 54 heures. Chez cinq d'entre eux l'on a administré le solvant, et chez sept la N-acétylcystéine intraveineuse (dose de charge préalable à la ventilation par l'oxygène à 100%: 150 $mg \cdot kg^{-1}$, et dose d'entretien: 20 $mg \cdot kg^{-1} \cdot h^{-1}$ en perfusion intraveineuse). Les animaux ont été traités de façon égale par ailleurs. Une évaluation physiologique de la circulation pulmonaire, des propriétés mécaniques, et des échanges gazeux, a été réalisée toutes les 6 heures. L'évaluation post-mortem a consisté en un examen macroscopique et en une pesée, suivis d'un examen au microscope optique et électronique. La N-acétylcystéine a protégé contre les effets de l'oxygène à 100%, à la fois selon les critères fonctionnels et structuraux. Le groupe sous N-acétylcystéine a développé moins d'augmentation de la résistance vasculaire pulmonaire, de la $Paco_2$ artérielle et du poids du poumon humide, alors que la compliance dynamique était plus élevée (différence significative). La N-acétylcystéine a également retardé le développement de relations ventilation-perfusion anormales et a été associée à une diminution de l'accumulation pulmonaire des globules blancs, avec moins de signes d'oedème alvéolaire et interstitiel. Quoique cette étude n'a pas tenté d'approcher les mécanismes de base par lesquels de fortes doses de NAC parentérale protègent le poumon, nous avons pu mettre en évidence de façon claire, à partir des mesures physiologiques, du poids du poumon et de l'examen microscopique, que la N-acétylcystéine avantage considérablement le poumon soumis à une respiration au long cours au moyen d'oxygène dans ce travail expérimental. Vu son acceptabilité pour l'utilisation humaine, NAC pourrait mériter une évaluaion comme agent thérapeutique dans des maladies humaines caractérisées par des lésions oxydatives.

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