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Effect of N-acetylcysteine on gas exchange after methacholine challenge and isoprenaline inhalation in the dog

O. Ueno, L-N. Lee, P.D. Wagner

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ABSTRACT: N-acetylcysteine (NAC) has antioxidant and possibly mucolytic properties. To determine whether NAC could be of benefit in acute bronchoconstriction induced by methacholine, 12 of 24 anaesthetized dogs (group 1) received NAC *i.v.* (loading dose 150 mg·kg^{-1}, then 20 mg·kg^{-1}·hr^{-1}). The other 12 (group 2) received diluent. Nebulized methacholine (1%) was then inhaled until arterial oxygen tension (Pao_2) fell to a mean of 5.5 kPa, after which isoprenaline 0.5% was inhaled in six dogs of each group to reverse bronchoconstriction. Over the next 3 h we measured total lung resistance, functional residual capacity (FRC), haemodynamic variables, and pulmonary gas exchange for respiratory and inert gases. After methacholine challenge, lung resistance increased and then fell similarly for both groups, but Pao_2 was higher in the NAC group (by 0.6–1.9 kPa) throughout the observation period. The ventilation-perfusion distribution measured by inert gas elimination also showed less abnormality in the NAC treated dogs over this time. Mucus was visible during post-mortem in the large airways in about half of the dogs in both groups, with no significant differences between them. These results show that NAC produces a measurable improvement in gas exchange following methacholine challenge (both with and without subsequent isoprenaline therapy) by mechanisms that remain to be determined.
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Dept of Medicine, University of California, San Diego, La Jolla, California.

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Since SCHEFFNER *et al.* [1] reported that N-acetylcysteine (NAC) has a mucolytic effect, inhaled NAC has been used occasionally to reduce the viscosity of mucus in the tracheobronchial tree. NAC may also have other actions, such as a being a free radical scavenger [2, 3], a substrate for the synthesis of glutathione [4, 5], and an accelerator of mucociliary clearance in the tracheobronchial tree [6, 7].

Several authors [8–11] have used NAC inhaled as an aerosol in smokers and patients with chronic obstructive lung disease, and reported reduced secretion of mucus or decreased viscosity. Others [12–14] have used an oral preparation of NAC and showed some beneficial effect in chronic bronchitis. Intravenously administered NAC has also been reported to be beneficial in man [15]. Because of these benefits, we gave oral NAC to patients with asthma [16] but could show no improvement over a 3-week period at a dose of 200 mg·day^{-1}. Because this dose may have produced too low a level of NAC in the respiratory tract, we decided to determine if high dose intravenous NAC could be shown to produce any physiological benefit in methacholine-induced bronchoconstriction in the dog.

We used the canine model of methacholine challenge and, in half the dogs, this was followed by an immediate isoprenaline inhalation to reverse the ensuing bronchoconstriction. NAC or inactive diluent was administered intravenously before methacholine challenge and we measured the resultant effects on pulmonary gas exchange, ventilation-perfusion ($\dot{V}A/\dot{Q}$) distribution, lung resistance, and functional residual capacity (FRC) over the subsequent 3 h. Finally, we assessed the presence of mucus in the large airways at post-mortem.

Methods

Twenty-four mongrel dogs (15–45 kg) were anaesthetized with intravenous injection of pentobarbital 30 mg·kg^{-1}, intubated with a Shiley 9 mm internal diameter (ID) endotracheal tube and connected to a Harvard ventilator. Tidal volume ranged from 12–15 ml·kg^{-1}, the frequency ranged between 15–20·min^{-1} to maintain the arterial carbon dioxide tension ($Paco_2$) between 4.7–5.6 kPa, and room air was breathed throughout. Positive end-expiratory pressure (PEEP) of 0.5 kPa was applied initially to prevent alveolar collapse. End-tidal CO_2 was monitored with a mass spectrometer (Perkin-Elmer MGA 1100, Pomona, CA) to document steady state conditions. A temperature probe was inserted into the oesophagus. The temperature was kept between 37.0 and 38.0°C, and the fluctuation in each experiment was

kept to within ±0.25°C by using ice packs or warming blankets as needed. A carotid artery was cannulated for monitoring of blood pressure and blood sampling. A Swan-Ganz catheter was floated into the pulmonary artery through an external jugular vein. Pressure in both vessels was monitored by Statham pressure transducers (Statham P23BB and P23ID, Hato Rey, PR). Two hind limb veins were cannulated and used for the infusion of: a) the multiple inert gas solution; and b) the solution of NAC or placebo, replacement fluid and supplemental doses of pentobarbital or pancuronium. Pentobarbital 60–120 mg was given when we observed body movement after painful stimuli, vigorous spontaneous ventilation or an increase in blood pressure. Pancuronium bromide 1–2 mg was given 15 min prior to each measurement after ensuring adequate anaesthesia. The right chest was opened and a Malecot catheter was inserted into the intrapleural cavity *via* the fifth interspace. It was connected to a Validyne differential pressure transducer (Validyne MP45, Northridge, CA) to measure the intrapleural pressure. Transpulmonary pressure was obtained by connecting the other side of the Validyne pressure transducer to the endotracheal tube.

PEEP was discontinued about 30 min before baseline measurements which were made after the stabilization of haemodynamics, end-tidal CO_2 and blood gases. Blood gas variables were measured by IL813 electrodes and an IL282 co-oximeter. Alveolar-arterial oxygen difference $P(A\text{-}a)o_2$ was calculated using the following formulae:

$$P(A\text{-}a)o_2 = PIo_2 - Paco_2 \cdot \{FIo_2 + (1-FIo_2)/R\} - Pao_2$$

$$R = F\bar{E}co_2 / \{0.2648 \cdot (1-F\bar{E}o_2 - F\bar{E}co_2) - F\bar{E}o_2\}$$

where PIo_2 is the inspired oxygen pressure, FIo_2 the concentration of the oxygen in the inspired air, $F\bar{E}o_2$ and $F\bar{E}co_2$, the mixed expired O_2 and CO_2 concentrations, respectively, which were measured by mass spectometer from the distal part of an expired gas mixing chamber attached to the expired gas port of a Harvard ventilator.

The ventilation-perfusion ($\dot{V}A/\dot{Q}$) distribution was measured by the multiple inert gas elimination technique [17–19]. The following outcome variables were used to compare the two groups of dogs: shunt (percentage of cardiac output to the non-ventilated alveoli and to areas with $\dot{V}A/\dot{Q} < 0.005$); mean $\dot{V}A/\dot{Q}_{bf}$ ($\dot{V}A/\dot{Q}$ at the mean of the blood flow distribution); log SDQ (second moment of the blood flow distribution on a logarithmic scale); mean $\dot{V}A/\dot{Q}_{vent}$ ($\dot{V}A/\dot{Q}$ at the mean of the ventilation distribution); log SDV (second moment of ventilation distribution on a logarithmic scale); dead space (percentage of total ventilation associated with non-perfused areas and areas with $\dot{V}A/\dot{Q}$ higher than 100) and percentage shunt plus perfusion of low $\dot{V}A/\dot{Q}$ areas ($\dot{V}A/\dot{Q} < 0.1$) [20].

Cardiac output was calculated by mass balance from the inert gas elimination data. Lung resistance was calculated by dividing the transpulmonary pressure by the flow rate (measured by a Fleisch No. 2 pneumotachograph). The elastic component of the pressure was subtracted electrically [21, 22]. FRC was measured by a closed circuit helium technique [23].

Dogs were randomly assigned into either the NAC or placebo group with twelve in each group. In each group, six subsequently received isoprenaline as well (see below).

After baseline measurements a loading dose of NAC (150 $mg \cdot kg^{-1}$) was given intravenously over 10 min, followed by the maintenance dose (20 $mg \cdot kg^{-1} \cdot min^{-1}$ in saline at 3 $ml \cdot kg^{-1} \cdot h^{-1}$) using an infusion pump. For the placebo group the same volume of diluent was used at the same saline infusion rate.

After the maintenance dose of NAC was started, 1% methacholine was given as an aerosol using a Bird respirator and nebulizer. The tidal volume and ventilatory frequency were kept similar to maintenance values. Methacholine inhalation was stopped when an airway inflation pressure of more than 2.0 kPa was needed to maintain the desired tidal volume and a mild cyanosis was found in the animal's tongue. Pao_2 was then measured, the final goal of methacholine challenge being a fall in Pao_2 of at least 5.3 kPa from baseline to less than 6.7 kPa. If the desired fall was not obtained, methacholine challenge was repeated. After this, systemic and pulmonary arterial pressures and lung resistance were measured, followed immediately in twelve of the 24 dogs by the inhalation of 0.5% isoprenaline for 1 min using the same system for methacholine inhalation.

The same variables as measured under baseline conditions were repeated at 30, 60, 90, 120 and 180 min after the isoprenaline inhalation.

All dogs were kept hydrated using normal saline at 10–12 $ml \cdot kg^{-1} \cdot hr^{-1}$. This represents fluid from all sources (NAC/placebo, inert gas solution and catheter irrigation). Control and NAC treated dogs received similar volumes of fluid.

After the last measurements, dogs were sacrificed with pentobarbital. The lungs were removed and the tracheobronchial tree was opened down to the level of the 6th generation to search for the presence and location of mucus or bronchial obstruction.

Values were expressed as mean±SE. The unpaired t-test was used to compare the baseline measurements of the two groups. A two-way analysis of variance with repeated measurements was used to compare the effects of NAC and diluent throughout the 3 h observation period after methacholine challenge. The chi-squared test was used to examine whether there were differences in the incidence of airway mucus at post-mortem between NAC and placebo groups.

Results

Baseline measurements

Prior to both methacholine challenge and NAC administration there were small but statistically significant differences in some parameters of gas exchange. In the twelve dogs not given isoprenaline, Pao_2 was 0.8 kPa higher and $P(A\text{-}a)o_2$ 0.9 kPa lower in the NAC group

(p=0.01 each). However, these differences were small and other parameters more directly reflecting the degree of $\dot{V}_A/\dot{Q}$ mismatch were no different between NAC and placebo groups ($Paco_2$, log SDQ, log SDV and perfusion of unventilated and low $\dot{V}_A/\dot{Q}$ regions; table 1 and fig. 1). In those given isoprenaline, Pao_2 averaged 12.8 and 11.7 kPa in the NAC and the placebo group, respectively ($p<0.05$) (table 2, fig. 2). $P(A\text{-}a)o_2$ averaged 2.5 and 4.0 kPa, respectively ($p<0.05$), and there was correspondingly less $\dot{V}_A/\dot{Q}$ inequality (mean log SDQ 0.44 in the NAC group and 0.67 in the placebo group) ($p<0.05$) (table 2, fig. 2).

to $\dot{V}_A/\dot{Q}$ region between 0.2–5 in every dog. We noted a small amount (6.7% mean) of ventilation in areas of high $\dot{V}_A/\dot{Q}$ ratios ($\dot{V}_A/\dot{Q}$ >10). Such areas were seen in most of the animals (20 out of 24).

Effects of methacholine challenge; subsequent recovery profiles

A single methacholine challenge was effective in lowering the Pao_2 to less than 6.7 kPa in 21 dogs, and only in 3 dogs were 2 or more challenges required. The

Table 1. – Gas exchange data before and after methacholine (mean±SE)

		Baseline	After Methacholine	30 min	60 min	90 min	120 min	180 min
Pao_2 kPa	NAC	13.0±0.2	5.1±0.3	9.1±0.6	10.9±0.6	11.3±0.6	11.3±0.6	11.2±0.6
	Control	12.2±0.2	5.7±0.1	8.5±0.4	10.0±0.4	10.3±0.4	10.2±0.4	10.4±0.4
$Paco_2$ kPa	NAC	5.0±0.1	6.0±0.2	5.7±0.3	5.3±0.2	5.2±0.2	5.0±0.1	5.0±0.2
	Control	5.0±0.1	6.0±0.2	5.8±0.2	5.4±0.2	5.2±0.2	5.2±0.2	4.9±0.2
$P(A\text{-}a)o_2$ kPa	NAC	1.6±0.3	7.3±0.7	4.5±0.5	3.3±0.3	3.1±0.5	3.1±0.4	3.2±0.5
	Control	2.5±0.1	8.2±0.3	5.8±0.3	4.6±0.3	4.5±0.3	4.4±0.3	4.5±0.3
Shunt %	NAC	0.7±0.1	—	7.8±2.1	5.5±2.5	4.9±2.6	5.0±2.7	5.8±3.0
	Control	1.4±0.2	—	14.0±3.1	8.5±1.7	6.7±1.6	6.1±1.5	6.4±1.2
Mean $\dot{V}_A/\dot{Q}_{bf}$	NAC	0.58±0.05	—	0.49±0.10	0.66±0.07	0.77±0.12	0.80±0.11	0.77±0.10
	Control	0.50±0.08	—	0.53±0.06	0.70±0.04	0.83±0.05	0.86±0.04	0.93±0.09
Log SDQ	NAC	0.51±0.04	—	1.18±0.19	0.75±0.05	0.67±0.06	0.64±0.06	0.66±0.06
	Control	0.61±0.06	—	1.45±0.16	0.93±0.04	0.81±0.07	0.82±0.07	0.80±0.07
Mean $\dot{V}_A/\dot{Q}_{vent}$	NAC	1.43±0.23	—	2.80±0.47	2.18±0.32	1.98±0.30	1.95±0.26	1.72±0.21
	Control	1.29±0.20	—	2.45±0.24	2.11±0.24	1.82±0.22	2.00±0.29	2.01±0.27
Log SDV	NAC	1.41±0.29	—	1.44±0.13	1.35±0.12	1.25±0.21	1.18±0.19	1.13±0.18
	Control	1.29±0.18	—	1.46±0.35	1.30±0.25	1.14±0.26	1.27±0.26	1.11±0.17
Dead-space %	NAC	44.2±1.9	—	40.5±1.4	43.3±1.5	42.8±1.9	42.8±2.8	44.4±2.3
	Control	45.8±1.5	—	43.0±1.7	43.8±1.8	45.5±1.6	43.3±2.6	43.0±1.2
Shunt plus low $\dot{V}_A/\dot{Q}$ perfusion %	NAC	1.4±0.7	—	12.7±4.3	5.7±2.6	5.0±2.6	5.0±2.7	5.9±3.0
	Control	1.4±0.2	—	23.9±5.6	9.2±1.6	6.9±1.7	6.7±1.1	6.9±1.2

Pao_2: arterial oxygen tension; $Paco_2$: arterial carbon dioxide tension; $P(A\text{-}a)o_2$: alveolar-arterial oxygen tension difference; $\dot{V}_A\dot{Q}$: ventilation-perfusion distribution; mean $\dot{V}_A/\dot{Q}_{bf}$, $\dot{V}_A/\dot{Q}_{vent}$: $\dot{V}_A\dot{Q}$ at mean of blood flow and ventilation distribution respectively; log SDQ, log SDV: second moment of blood flow and ventilation distribution on a logarithmic scale, respectively; NAC: N-acetylcysteine.

There were no significant differences in baseline haemodynamics, $Paco_2$, other parameters of the $\dot{V}_A/\dot{Q}$ distribution, lung resistance or FRC between NAC and placebo groups (tables 1–4). Compared to the severe hypoxaemia (Pao_2 5.5 kPa similar in both groups, table 1) produced subsequently by methacholine challenge, we believe these small differences are of little importance.

The $\dot{V}_A/\dot{Q}$ distribution was unimodal and confined

necessary methacholine inhalation times were statistically the same in the placebo groups at 95 (NAC) and 112 (placebo) seconds each. Pao_2 in the NAC and placebo groups after methacholine challenge were no different, averaging 5.4 and 5.7 kPa, respectively, (tables 1 and 2, figs 1 and 2).

Systemic arterial, pulmonary arterial and pulmonary capillary wedge pressures did not change throughout the study, and there were no significant differences between

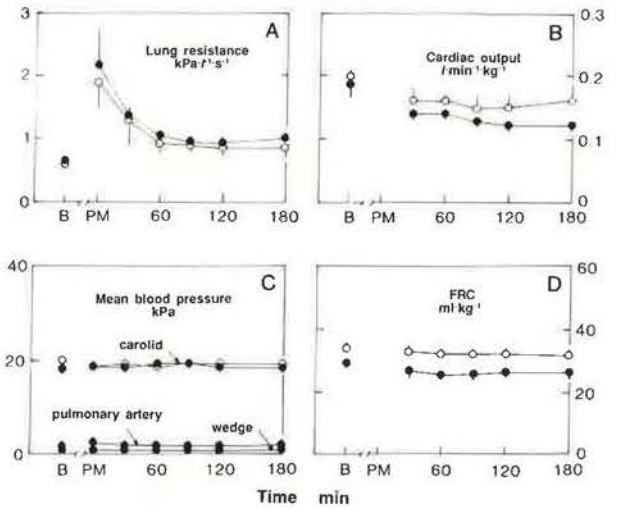

Fig. 1. – Changes in lung resistance, cardiac output, haemodynamics, and functional residual capacity (FRC) after methacholine challenge (mean±SE). B: prior to methacholine and N-acetylcysteine (NAC); PM: within 2 min after methacholine. Lung resistance and vascular pressures were no different throughout the study, but cardiac output and FRC were slightly higher in the NAC treated group (○) than in controls (●)

NAC and placebo groups (tables 3 and 4, figs 3 and 4). In dogs not given isoprenaline, cardiac output fell after methacholine challenge and remained essentially constant thereafter. NAC in these dogs somewhat attenuated this fall in cardiac output (table 3, fig. 3). In dogs given isoprenaline, cardiac output was increased at 30 min post-challenge, falling gradually thereafter, so that by 180 min the average value was slightly lower than at baseline (table 4, fig. 4). In these dogs, NAC had no effect.

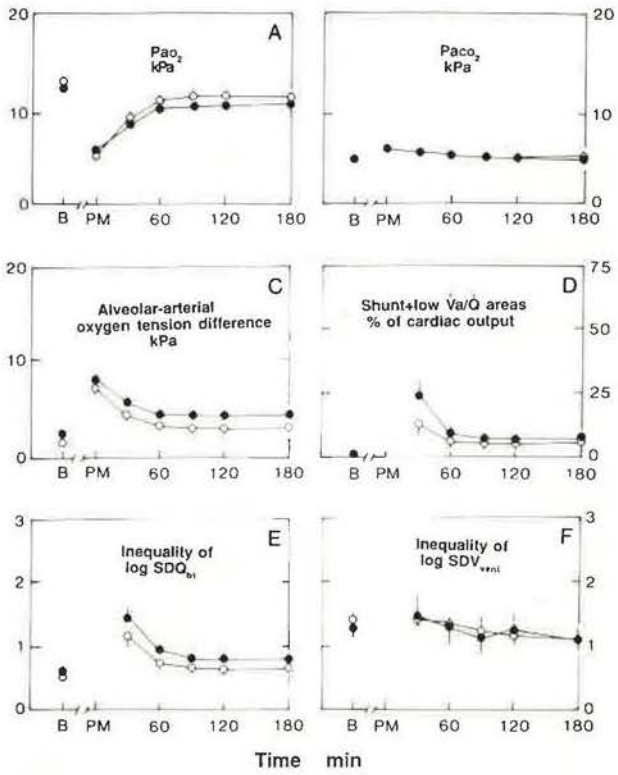

Fig.2. – Changes in gas exchange variables after methacholine (mean±SE). B: prior to methacholine and N-acetylcysteine (NAC); PM: within 2 min after methacholine. Very small differences in arterial oxygen tension (Pao_2) and alveolar-arterial oxygen tension difference ($P(A\text{-}a)o_2$) but not in the parameters of the ventilation-perfusion ($\dot{V}_A/\dot{Q}$) distribution were seen at B. Following methacholine, $\dot{V}_A/\dot{Q}$ mismatching and hypoxaemia were less severe in NAC treated dogs (○) than in controls (●).

Table 2. – Gas exchange data before and after methacholine and isoprenaline (mean±SE)

		Baseline	After Methacholine	30 min	60 min	90 min	120 min	180 min
Pao_2 kPa	NAC	12.8±0.4	5.7±0.4	9.0±0.6	10.9±0.4	11.3±0.4	11.2±0.4	11.5±0.5
	Control	11.7±0.3	5.6±0.3	7.3±0.7	9.7±0.6	10.4±0.6	9.5±0.7	9.6±0.5
$Paco_2$ kPa	NAC	5.0±0.2	5.9±0.2	6.6±0.4	5.7±0.3	5.2±0.2	5.1±0.2	4.8±0.2
	Control	5.0±0.4	6.0±0.2	6.8±0.3	6.0±0.2	5.5±0.2	5.4±0.1	5.2±0.2
$P(A\text{-}a)o_2$ kPa	NAC	2.5±0.2	8.5±0.7	3.7±0.5	3.2±0.2	3.6±0.5	3.7±0.5	2.9±0.3
	Control	4.0±0.4	8.9±0.4	5.8±0.3	4.7±0.5	5.2±0.7	5.3±0.9	5.3±0.6
Shunt %	NAC	1.7±0.6	—	8.6±2.4	5.0±1.5	4.2±1.3	4.1±1.2	3.6±1.1
	Control	3.2±0.8	—	22.9±5.9	10.0±3.2	11.1±4.2	11.6±3.6	12.2±3.8
Mean $\dot{V}_A/\dot{Q}_{bf}$	NAC	0.60±0.05	—	0.35±0.05	0.58±0.06	0.72±0.12	0.75±0.14	0.88±0.16
	Control	0.71±0.07	—	0.40±0.06	0.69±0.07	0.90±0.07	0.74±0.06	0.82±0.05
Log SDQ	NAC	0.44±0.04	—	1.06±0.15	0.72±0.06	0.64±0.07	0.57±0.07	0.56±0.05
	Control	0.67±0.06	—	1.56±0.25	0.99±0.19	0.80±0.07	0.89±0.16	0.79±0.09
Mean $\dot{V}_A/\dot{Q}_{vent}$	NAC	1.54±0.29	—	1.93±0.33	2.00±0.18	1.83±0.33	1.55±0.19	1.41±0.18
	Control	1.27±0.14	—	2.62±0.38	2.06±0.31	2.16±0.34	1.98±0.27	1.94±0.36
Log SDV	NAC	1.38±0.35	—	1.60±0.13	1.56±0.09	1.35±0.11	1.26±0.14	0.87±0.12
	Control	0.94±0.12	—	1.27±0.13	1.12±0.14	1.01±0.11	1.07±0.16	1.00±0.15
Dead-space %	NAC	48.8±1.8	—	45.9±2.1	39.6±2.3	39.8±4.3	44.9±1.8	44.7±2.2
	Control	48.0±1.9	—	46.4±2.9	44.8±2.3	42.5±2.5	47.0±2.8	45.4±1.7
Shunt plus low $\dot{V}_A/\dot{Q}$ perfusion %	NAC	1.7±0.6	—	18.8±6.0	5.6±1.8	4.4±1.4	4.1±1.2	3.6±1.5
	Control	3.5±0.9	—	31.9±7.3	12.5±5.4	11.1±4.2	12.0±3.9	12.2±3.9

Legend as for table 1.

Table 3. – Haemodynamics, lung resistance and lung volume before and after methacholine (mean±SE)

		Baseline	After Methacholine	30 min	60 min	90 min	120 min	180 min
Systemic arterial pressure kPa	NAC	20.0±0.7	18.5±0.8	19.2±0.7	18.8±1.3	19.3±0.8	19.2±0.8	19.1±0.8
	Control	18.4±0.5	18.3±0.8	18.5±0.5	19.1±0.4	19.3±0.5	18.3±0.3	18.3±0.4
Pulmonary arterial pressure kPa	NAC	1.5±0.1	2.5±0.3	1.9±0.2	1.7±0.2	1.7±0.2	1.8±0.2	2.0±0.3
	Control	1.7±0.1	2.5±0.2	1.9±0.1	1.9±0.2	2.1±0.2	2.1±0.2	2.3±0.2
Pulmonary wedge pressure kPa	NAC	0.6±0.1	0.6±0.1	0.6±0.1	0.7±0.2	0.7±0.2	0.8±0.3	0.6±0.2
	Control	0.7±0.1	0.9±0.1	0.7±0.1	0.8±0.1	0.9±0.1	0.9±0.1	0.9±0.2
Cardiac output $l \cdot min^{-1} \cdot kg^{-1}$	NAC	0.20±0.01	—	0.16±0.02	0.16±0.01	0.15±0.02	0.15±0.02	0.16±0.02
	Control	0.19±0.02	—	0.14±0.01	0.14±0.01	0.13±0.01	0.12±0.01	0.12±0.01
Lung resistance $kPa \cdot l^{-1} \cdot s^{-1}$	NAC	0.61±0.10	1.87±0.39	1.31±0.36	0.93±0.15	0.90±0.14	0.86±0.14	0.88±0.15
	Control	0.66±0.05	2.16±0.53	1.39±0.11	1.05±0.09	0.96±0.04	0.93±0.05	1.02±0.12
FRC $ml \cdot kg^{-1}$	NAC	33.9±1.9	—	32.8±1.9	32.2±1.8	32.1±2.0	32.1±1.9	31.7±2.0
	Control	29.4±1.2	—	26.8±2.5	25.4±1.6	25.7±1.9	26.2±1.9	26.3±2.0

FRC: functional residual capacity; NAC: N-acetylcysteine

Table 4. – Haemodynamics, lung resistance and lung volume before and after methacholine and isoprenaline (mean±SE)

		Baseline	After Methacholine	30 min	60 min	90 min	120 min	180 min
Systemic arterial pressure kPa	NAC	19.6±0.8	19.7±0.8	18.8±0.8	19.6±0.9	19.3±0.8	19.4±0.5	18.8±0.5
	Control	19.2±0.8	17.1±2.0	18.4±0.4	19.2±0.4	19.2±0.7	19.2±0.7	19.3±0.7
Pulmonary arterial pressure kPa	NAC	2.3±0.4	2.8±0.2	2.2±0.2	2.1±0.1	2.1±0.1	2.3±0.1	2.3±0.1
	Control	2.1±0.2	3.0±0.3	2.4±0.1	2.2±0.2	2.3±0.2	2.4±0.2	2.6±0.2
Pulmonary wedge pressure kPa	NAC	0.7±0.1	—	0.7±0.1	0.8±0.1	0.9±0.1	1.0±0.2	1.1±0.2
	Control	0.7±0.1	—	0.8±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.8±0.1
Cardiac output $l \cdot kg^{-1} \cdot min^{-1}$	NAC	0.17±0.01	—	0.21±0.02	0.18±0.02	0.16±0.01	0.16±0.02	0.14±0.02
	Control	0.18±0.02	—	0.19±0.02	0.16±0.01	0.14±0.01	0.15±0.01	0.16±0.01
Lung resistance $kPa \cdot l^{-1} \cdot s^{-1}$	NAC	0.47±0.05	1.60±0.27	0.76±0.12	0.65±0.07	0.67±0.07	0.64±0.07	0.63±0.06
	Control	0.56±0.08	1.28±0.35	0.83±0.13	0.74±0.12	0.73±0.12	0.66±0.10	0.69±0.11
FRC $ml \cdot kg^{-1}$	NAC	28.5±2.1	—	26.9±1.8	28.4±1.9	27.2±2.0	26.9±2.1	25.9±1.7
	Control	25.0±2.5	—	23.3±2.6	23.5±2.6	22.9±2.4	25.2±3.3	22.6±2.6

FRC: functional residual capacity; NAC: N-acetylcysteine

Lung resistance increased abruptly after the methacholine challenge and averaged 1.7 $kPa \cdot l^{-1} \cdot s^{-1}$ in both the NAC and placebo groups (tables 3 and 4, figs 3 and 4). By 30 min resistance was only slightly higher than normal, but it remained relatively constant thereafter. There was no significant difference between NAC and placebo groups (tables 3 and 4, figs 3 and 4).

In the dogs not given isoprenaline, FRC was slightly less throughout the study in the placebo group, for unaccountable reasons. Methacholine challenge did not influence FRC in either NAC or placebo groups (fig. 3). FRC after challenge and isoprenaline treatment did not change from the baseline level in either group, and there was no significant difference between NAC and placebo groups (fig. 4).

Both in dogs given isoprenaline and in those not given the bronchodilator, Pao_2 began to recover rapidly over the first 60 min post-challenge, but remained relatively constant thereafter. With or without isoprenaline, Pao_2 was significantly higher ($p<0.01$) in the NAC group than in the placebo group from 30 min after challenge onwards (tables 1 and 2, figs 1 and 2). $Paco_2$ increased at 30 min and decreased gradually thereafter. No significant differences in $Paco_2$ between NAC and placebo groups were found whether or not isoprenaline was used (tables 1 and 2, figs 1 and 2).

$P(A\text{-}a)o_2$ increased after methacholine challenge in all 24 dogs, decreased rapidly by 60 min, and remained almost constant thereafter. Values after challenge were always higher than baseline values, and were significantly

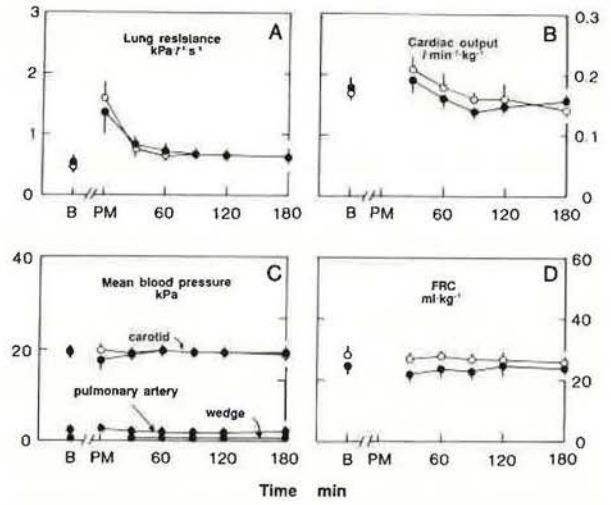

Fig. 3. – Changes in lung resistance, cardiac output, haemodynamics and functional residual capacity (FRC) after methacholine challenge followed by inhalation of isoprenaline (mean±SE). B: prior to methacholine and N-acetylcysteine (NAC); PM: within 2 min after methacholine. There were no significant differences between the NAC treated group (◯) and control (●) throughout the study in any of these variables.

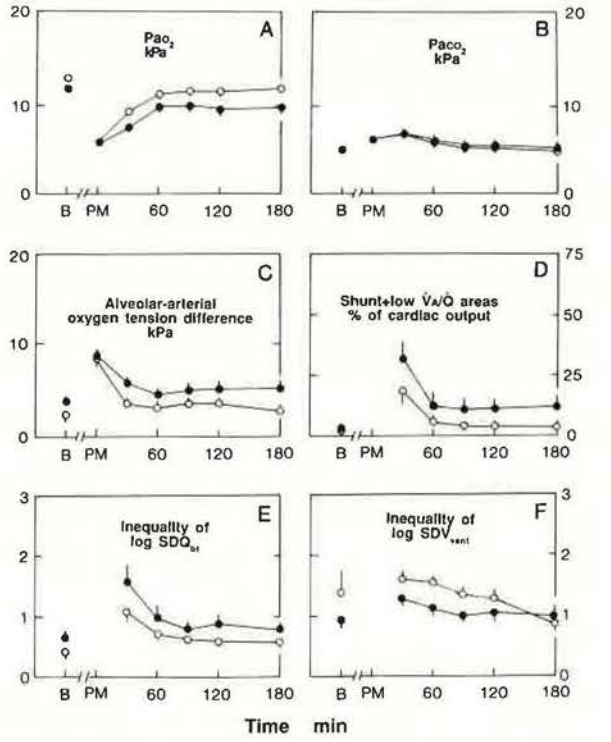

Fig. 4. – Changes in gas exchange variables (mean±SE). B: prior to methacholine and N-acetylcysteine (NAC); PM: within 2 min after methacholine. There were small but significant differences in the arterial oxygen tension (Pao_2), alveolar-arterial oxygen tension difference ($P(A\text{-}a)o_2$) and second moment of blood flow distribution on a logarithmic scale (log SDQ_{bf}) in the baseline values between the NAC treated group (◯) and control (●). After methacholine challenge and isoprenaline inhalation Pao_2 and second moment of ventilation on a logarithmic scale (log SDV_{vent}) were significantly higher in the NAC treated group, and $P(A\text{-}a)o_2$, shunt±low ventilation-perfusion distribution ($\dot{V}A/\dot{Q}$ <0.1) and log SDQ_{bf} were significantly lower throughout the observation period.

higher in the placebo group than in the NAC group ($p<0.01$) both with and without isoprenaline. Post-challenge differences between the two groups were always higher than baseline differences (tables 1 and 2, figs 1 and 2).

There were differences in $\dot{V}A/\dot{Q}$ matching between the two groups. The percentage of shunt, and combined perfusion of unventilated and low $\dot{V}A/\dot{Q}$ areas increased after challenge (tables 1 and 2, figs 1 and 2) and then decreased thereafter. Mismatching indices were consistently and significantly higher in the placebo group than in the NAC group ($p<0.01$), both with and without isoprenaline. The mean $\dot{V}A/\dot{Q}_{bf}$ decreased at 30 min and then returned toward baseline levels, and there were no significant differences between the two groups (tables 1 and 2). The mean $\dot{V}A/\dot{Q}_{vent}$ increased at 30 min and then decreased gradually in all dogs. Log SDQ was significantly higher in the placebo group at all times (tables 1 and 2, figs 1 and 2). In dogs not given isoprenaline, log SDV was not affected either by challenge or by NAC. After methacholine plus isoprenaline, log SDV was significantly higher ($p<0.01$) early in the NAC group after challenge, but not over the last 1 h of observation (table 2, fig. 2). There was no difference in the percentage of the dead space between the NAC and placebo groups, whether or not isoprenaline was given, and this variable remained constant throughout the entire experiment (tables 1 and 2).

Post-mortem data

Post-mortem examination revealed the presence of dark reddish, congested areas in the dependent parts of the lung in every dog. Sponge-like, overinflated areas were also found, especially in the upper lobes. Foamy or slightly tenacious mucus was found in the tracheobronchial tree in 10 dogs (5 out of 12 in the NAC and 5 out of 12 in the placebo group), and in 4 dogs (1 in the NAC and 3 in the placebo group) the mucus caused marked obstruction of the bronchial tree at generations 2–5. By chi-squared analysis the incidence of mucus in the airways was no different between NAC and control dogs. Atelectatic areas were seen distal to areas of bronchial obstruction. The amount of recoverable mucus was small (less than 2 ml), except in one dog of the placebo group in which we found 20 ml of serous mucus which did not cause bronchial obstruction or atelectasis.

In both the NAC and the placebo groups, dogs in which large airway obstruction by mucus was evident also had a high percentage of shunt (28.5±12.6% SEM) as well as perfusion to areas of low $\dot{V}A/\dot{Q}$ ratios, and the shunt remained high throughout the 180 min period (15.9±8.9% at 180 min). In two dogs in the placebo group, perfusion to areas of low $\dot{V}A/\dot{Q}$ also persisted up to the 180-min measurement. In contrast, dogs which did not have bronchial obstruction by mucus had a lowered percentage of shunt (10.3±1.6% at 30 min) which decreased further by 180 min to 5.2±1.0%.

Discussion

Following methacholine challenge, both with and without an immediate isoprenaline inhalation, we found faster and more complete recovery of Pao_2 and $\dot{V}A/\dot{Q}$ distribution in dogs given NAC than in control animals. This improvement in pulmonary gas exchange was not associated with decreased large airway mucus accumulation in the NAC group, as judged visually and volumetrically at post-mortem in airway generations 2–6.

There were small but significant baseline differences in some parameters of gas exchange between NAC and placebo groups. The Pao_2 was slightly lower in the placebo group than in the NAC group, although both were within normal limits [24]. Baseline log SDQ and other parameters (tables 1 and 2) were similar in NAC and placebo groups for dogs not given isoprenaline. However, log SDQ was slightly different between NAC and placebo groups of dogs who subsequently received the bronchodilator. Values of log SDQ were, however, within the normal range [24]. Moreover, baseline percentages of shunt (and perfusion of shunt plus low $\dot{V}A/\dot{Q}$ areas), *i.e.* the most important regions for impaired pulmonary O_2 exchange were low, and were not significantly different between NAC and placebo groups whether or not isoprenaline was later given (tables 1 and 2). Calculated baseline $P(A\text{-}a)o_2$ was slightly different between NAC and placebo groups, but when compared with post-challenge values, these differences were small (tables 1 and 2, figs 1 and 2).

We think that these small baseline differences are of minimal importance because we were able to reach similar levels for Pao_2, $Paco_2$ and $P(A\text{-}a)o_2$ immediately after the methacholine challenge, thus assuring the same starting point for the majority of this study. In addition, the high lung resistance values measured immediately after the methacholine challenge were no different between NAC and placebo treated dogs. We have no $\dot{V}A/\dot{Q}$ data immediately after the challenge due to lack of steady state conditions [17].

By 30 min after methacholine, at the time of the first post-challenge inert gas measurements, $\dot{V}A/\dot{Q}$ distributions were perturbed more in dogs receiving placebo, irrespective of isoprenaline therapy. Figures 1 and 2 showing the shunt and perfusion of low $\dot{V}A/\dot{Q}$ regions over the entire study also illustrate these points.

Effects of isoprenaline

We are not certain whether a 1 min inhalation of 0.5% isoprenaline is enough to fully reverse the bronchoconstriction after methacholine challenge, nor did we measure the effects of 0.5% isoprenaline inhalation *per se* on the $\dot{V}A/\dot{Q}$ distribution, although some effect can be predicted from previous studies [25, 26]. However, by giving the same amount of isoprenaline to both groups, they remain comparable. Note from figures 3 and 4 that lung resistance was higher in dogs who did not receive isoprenaline, consistent with at least partial reversal of bronchostriction by the drug. Moreover, in both groups lung resistance recovered to near-baseline levels and FRC was no different from baseline at the 30 min measurement, consistent with reversal of major airways obstruction.

Variability of gas exchange response to methacholine

Our 30-min $\dot{V}A/\dot{Q}$ measurement revealed that in addition to the main $\dot{V}A/\dot{Q}$ mode centred near a $\dot{V}A/\dot{Q}$ ratio of 1.0, in 10 animals (3 in the NAC and 7 in the placebo group) there was a low $\dot{V}A/\dot{Q}$ mode, and in 11 animals (9 in the NAC and 2 in the placebo group) there was a high $\dot{V}A/\dot{Q}$ mode. Two dogs in the placebo group showed widely distributed $\dot{V}A/\dot{Q}$ inequality. In all animals there was some shunt present. These changes are consistent with prior data. Thus, methacholine is well-known to produce an abnormal $\dot{V}A/\dot{Q}$ distribution, often with a low $\dot{V}A/\dot{Q}$ mode, presumably by bronchoconstriction and mucus obstruction [25]. Isoprenaline may worsen the inequality when it is inhaled after the methacholine challenge as reported by RODRIGUEZ-ROISIN *et al.* [26]. However, RUBINFELD *et al.* [25] found that isoprenaline inhalation after methacholine challenge resulted in a widely dispersed unimodal $\dot{V}A/\dot{Q}$ distribution. Our 30-min data revealed even more diverse types of $\dot{V}A/\dot{Q}$ inequality. It is evident that over a variety of such $\dot{V}A/\dot{Q}$ patterns NAC appears to provide more rapid resolution of the physiological abnormalities in gas exchange.

Mechanism of action of NAC

We do not know how NAC was of benefit in the present study but we offer some possibilities. A well-known action of NAC is to break down the disulphide cross-linkage between protein and DNA in the mucus by its free sulphydryl group [27]. GRASSI *et al.* [15] used NAC intravenously in patients with chronic bronchopulmonary diseases and noted improvement when compared with the effect of oral or topical administration. COTGREAVE *et al.* [28] reported that orally administered NAC did not appear in pulmonary lavage fluid, which may explain the result of Grassi's study. The current study employed intravenous doses at levels similar to those of GRASSI *et al.* [16] and our results are in accord with theirs.

Another possible action of NAC is to decrease the production or secretion of mucus by secretory cells. COTGREAVE *et al.* [28] proposed the hypothesis that NAC depressed mucus production by increasing the synthesis of glutathione [4, 5]. Methacholine is a parasympathomimetic agent which can increase mucus production by stimulating secretory cells [29]. Thus NAC may have decreased methacholine-induced hypersecretion of mucus.

The action of NAC as a free radical scavenger was reported by BERGSTRAND *et al.* [2] and MOLDEUS *et al.* [3]. This might prevent cellular damage from free radicals produced by inflammatory cells. We do not know if methacholine produces inflammatory changes in the

tracheobronchial tree, although we did find capillary dilatation in the tracheobronchial wall in every dog at autopsy. No histological examination of the airways was performed.

Yet another possible action of NAC is the facilitation of mucociliary movement. Mucociliary clearance was not measured in our study. Probably all of the actions mentioned above work together to some extent to decrease mucus accumulation in the tracheobronchial tree.

In conclusion, intravenous NAC pretreatment followed by its continuous infusion led to more rapid and complete recovery of gas exchange abnormalities caused by methacholine inhalation than did administration of placebo. This was observed whether or not methacholine challenge had been followed by isoprenaline inhalation. Which of several potential mechanisms is responsible needs to be determined.

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Effet de la N-acétylcystéine sur les échanges gazeux après provocation à la métacholine et inhalation d'isoprénaline chez le chien. O. Ueno, L.M. Lee, P. Wagner.

RÉSUMÉ: La N-acétylcystéine a des propriétés anti-oxydantes et peut-être mucolytiques. Pour déterminer si la NAC pouvait être utile dans la bronchoconstriction aiguë induite par la métacholine, nous avons administré à 12 de 24 chiens anesthésiés (groupe 1), de la NAC par voie intraveineuse (dose de

charge: 150 $mg \cdot kg^{-1}$, puis 20 $mg \cdot kg^{-1} \cdot h^{-1}$). Les 12 autres chiens (groupe 2) ont reçu le produit diluant. La métacholine à 1% été ensuite nébulisée et inhalée jusqu'à ce que la Pao_2 artérielle tombe à une moyenne de 5.5 kPa, après quoi de l'isoprénaline à 0.5% a été inhalée chez 6 chiens de chaque groupe pour corriger la bronchoconstriction. Au cours des 3 heures suivantes, nous avons mesuré la résistance pulmonaire totale, la capacité résiduelle fonctionnelle, des variables hémodynamiques, ainsi que les échanges gazeux pulmonaires pour les gaz respiratoires et inertes. Après provocation à la métacholine, la résistance pulmonaire augmente et elle chute ensuite de façon similaire pour les deux groupes, mais la Pao_2 artérielle est plus élevée dans le groupe NAC (0.6–1.9 kPa) pendant toute la période d'observation. La distribution de la ventilation et de la perfusion, mesurée par élimination d'un gaz inerte, a démontré moins d'anomalies dans le groupe des chiens traités par la NAC pendant cette période. On a trouvé en post-mortem, dans les grosses voies aériennes, du mucus chez environ la moitié des chiens des deux groupes, sans différence significative entre eux. Ces résultats montrent que la NAC provoque une amélioration mesurable des échanges gazeux après provocation à la métacholine, à la fois avec et sans traitement subséquent à l'isoprénaline, par des mécanismes qui restent à déterminer.
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