Effect of oral N-acetylcysteine (NAC) on volume and albumin content of respiratory tract fluid but not on epithelial secretory cell number in "smoking" rats

N. Robinson, R. Brattsand, M. Dahlbäck

ABSTRACT: This study was designed to look at the effect of N-acetylcysteine (NAC) on epithelial secretory cells and the respiratory tract fluid volume and albumin content from the lower airways of "bronchitic" rats. Rats were exposed either to tobacco smoke (TS), TS and NAC, or NAC alone. TS caused a significant increase in epithelial secretory cell number which was not reduced by concomitant NAC administration; NAC alone had no effect on cell numbers. TS increased respiratory tract fluid volume and albumin content by a small but non-significant amount, whereas TS and NAC increased the volume and albumin content by a greater and significant amount; NAC alone was also shown to significantly increase both fluid volume and albumin content. Eur Respir J., 1990, 3, 304-310.

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The rat has been shown in a number of "bronchitic" models to react to inhaled tobacco smoke (TS) with the hyperplasia of epithelial secretory cells [6, 7], and by the hypersecretion of mucus from submucosal glands in the upper trachea and larynx [8, 9]. This report gives details of the effect of TS, NAC and TS+NAC on epithelial secretory cell (goblet cell) number and on respiratory tract fluid (RTF) volume and its albumin content, collected from the lower airways of rats.

Materials and methods

A total of seven experiments was performed with male albino, specific pathogen free, Sprague-Dawley rats of 175-200 g. Experiments 1-3 were concerned with the effects of TS and TS+NAC on secretory cell numbers, whilst experiment 4 looked at the effects of NAC alone on secretory cell numbers. Experiments 5 and 6 looked at the effects of TS and TS+NAC on RTF and albumin from the lower airways whilst experiment 7 looked at the effect of NAC alone on RTF and albumin. Different animals were used for each experiment and were randomly allocated in equal numbers to control and experimental groups for each experiment (n values are given in results). Animals were housed in clean animal rooms and, except during periods of exposure, were given standard rat pellets and tap water ad libitum. Animals were weighed every two days during the experimental period.

NAC was given to experimental animals as a 1% solution in drinking water; the pH was adjusted to 7.0 with the solution being changed every two days. At the end of 48 h, approximately 5% of the NAC solution had been oxidised to the product N`N`-diacetylcysteine; the addition of 1.25 mM edetic acid (EDTA) to the 1% NAC solution did not alter its rate of degradation. The calculated daily dose of NAC consumed by each rat was equivalent to 960 mg·kg⁻¹ body weight and, except for an initial decrease, was drunk in the same volume as the tap water of the control rats. Dosing of rats with NAC...
N-ACETYLCYSTEINE IN SMOKE INDUCED BRONCHITIS

Rats were individually restrained in tubes attached to a Battelle chamber [10], each rat being exposed to puffs of TS interspersed with fresh air in a nose only fashion. Rats were exposed to TS for 1 h each day for a total of 14 consecutive days. Control rats were restrained in identical chambers but only exposed to fresh air.

Kentucky high-yield reference (2R1) cigarettes were used on a rotating wheel which was moved by an electrical step motor (fig. 1), each cigarette being lit in turn by a heated metal coil. On the reverse side of the smoking wheel a tube with a rubber gasket was firmly attached to ensure a gas-tight fit. The tube was then connected to a 4-way Y-piece where the entry of fresh air instead of TS could occur when required. A homemade centrifugal fan was attached to the bottom of the Y-piece making it possible to draw air through the cigarette into the system and then to the chambers; the fan was rotated at constant speed at all times. To each side of the tube connected to the Y-piece a solenoid valve with a scissor clamping device was mounted making it possible to control the intervals of fresh air and smoke.

Each cigarette was smoked to a standard stub length of 2 mm in about 30 puffs each lasting 5 s at a flow rate of 0.2-0.4 l/min, each puff being interspaced with 10 s of fresh air at a flow rate of 2 l/min. Exhaust air was drawn from the chambers with a flow rate of 2 l/min. The whole smoking generation system was controlled by computer (fig. 1).

Throughout the whole exposure period, smoke was drawn at a constant flow rate of 0.1 l/min from a spare port of the Battelle chamber onto a filter (Schleicher & Schull, 50 mm diam, AE91 pore size 0.8 μm). The weight of the smoke particles collected on filters per unit time gave an indication of the smoke concentration (mg/l³ air) to which the rats were exposed.

SECRETORY CELL NUMBERS

Rats were killed by asphyxiation in carbon dioxide 24 h after the last TS exposure, and the lungs were inflated to constant volume with neutral buffered formalin for 24 h. The left lung was prepared for light microscopy. Paraffin sections (6 μm thick) were cut to expose the main axial pulmonary bronchi with its lateral branches. Sections were stained with Alcian blue (pH 2.5) and periodic acid-Schiff reagent. Slides were coded so that their identity was unknown to the observer, and the number of stained mucous containing epithelial secretory cells determined in at least 6 mm of epithelium from specified areas. In addition, measurements of epithelial thickness were made on the upper trachea.

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Rats were anaesthetized with sodium pentobarbitone (30 mg·kg⁻¹, i.p.) 24 h after the last TS exposure, were injected i.v. with 1 mg FITC-labelled rat albumin to act as a macromolecular tracer, and had their lower trachea cannulated. A small piece of pre-weighed absorbent paper...
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Absorbed fluid on the papers was eluted into 1 ml distilled water. Its total albumin content was estimated from the fluorescence spectroscopy determined FITC-labelled albumin concentration, assuming its FITC-albumin to total albumin ratio to be the same as that found in the rat's plasma. The median albumin concentration for the ten collection periods was determined. During the collection period, rats did not breath humidified air and, therefore, a certain degree of water evaporation is likely to have occurred; thus volumes given in the results section are for comparative purposes only.

Statistics
In experiments 1–4 the data were normally distributed, and mean values are given and groups compared using Student's separate variance t-test for unpaired data. In experiments 5–7 the data was not normally distributed and median values are given and groups compared using the Mann-Whitney U-test. Statistically significant difference was taken to be p<0.05 unless otherwise stated.

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Results

TS-exposed rats showed an initial drop in weight gain and never recovered to control weights, their final body weight being approximately 10% less than the controls. On removal from the Battelle chambers the rats were subdued and showed signs of piloerection and excess salivation compared to controls, but quickly recovered. NAC-treated rats showed an initial drop in weight gain but quickly recovered to control weights: this drop in weight gain was thought to be caused by an initial reluctance of the rats to drink the NAC solution. NAC-treated rats showed no other ill-effects and NAC treatment did not alter the weight gain or immediate physical effects of TS-exposed rats.

Morphological investigations

The effect of TS on the morphological parameters studied are exemplified in Table 1 with results from experiment 1. In the trachea TS enhanced the epithelial thickness by about 30% (p<0.001). The epithelial mucous cell number was elevated in the axial and proximal lateral bronchi of the lung. The greatest rise was observed along the axial bronchi, where the secretory cell number was doubled (p<0.05) compared with the control rats. Along the carinae of the proximal lateral bronchus and the lateral bronchus the epithelial cell number was about 50% higher (p<0.01–0.05) than in the control group. However, we could not observe any TS-induced change of the low secretory cell numbers in the distal lateral bronchi (Table 1).

Based on these findings the morphological investigation in experiments 2–4 was concentrated on the counting of secretory cells per 6 mm axial bronchus. Results of the two further experiments with TS are given in Table 2. The TS-induced hyperplasia was reproducible as the epithelial secretory cell number increased by at least 100%. In these two experiments the specific effect of TS on acidic glycoprotein and neutral glycoprotein

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N-ACETYLCYSTEINE IN SMOKE INDUCED BRONCHITIS

2. Effect of TS, TS+NAC and NAC alone on secretory cell number per mm of axial bronchus

<table>
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<tr>
<th>TS concn Controls</th>
<th>TS</th>
<th>TS+NAC</th>
<th>NAC</th>
</tr>
</thead>
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<tr>
<td>mg/l 100</td>
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</tr>
<tr>
<td>6.03</td>
<td>28±4* (87%)</td>
<td>31±4* (107%)</td>
<td>15±2</td>
</tr>
<tr>
<td>11±2</td>
<td>45±7** (275%)</td>
<td>33±3** (136%)</td>
<td>12±4</td>
</tr>
<tr>
<td>5.72</td>
<td>20±1 (0%)</td>
<td>41±6** (242%)</td>
<td>14±2</td>
</tr>
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The effect of oral NAC on the TS-induced hyperplasia of epithelial secretory cells is summarized in tables 1 and 2. In both experiments, NAC did not significantly affect the number of secretory cells either in the control or in the TS-treated groups (table 1) or at the other bronchial levels.

Fig. 3. Effect of oral NAC on the TS-induced hyperplasia of epithelial secretory cells. Results are mean±SEM and % increase from control in parentheses. *: p<0.05; **: p<0.01 compared to controls; TS: tobacco smoke; NAC: N-acetylcysteine.
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Keywords: Airways; albumin; bronchitis; cigarette smoke; epithelial secretory cells; N-acetylcysteine; respiratory tract fluid.

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N-Acetylcysteine in smoke induced bronchitis

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Based on these findings the morphological investigation in experiments 2–4 was concentrated on the counting of secretory cells per 6 mm axial bronchus. Results of the two further experiments with TS are given in table 2. The TS-induced hyperplasia was reproducible as the epithelial secretory cell number increased by at least 100%. In these two experiments the specific effect of TS on acidic glycoprotein and neutral glycoprotein

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Table 2. — Effect of TS, TS+NAC and NAC alone on secretory cell number per mm of axial bronchus

<table>
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<th>Experiment series</th>
<th>n</th>
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<td>1</td>
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<td>0.72</td>
<td>15±2</td>
<td>31±4* (+107%)</td>
<td>28±4* (+87%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.72</td>
<td>12±4</td>
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</tr>
<tr>
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<td>5</td>
<td>0.63</td>
<td>14±2</td>
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<tr>
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<td>6</td>
<td>0.63</td>
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The effect of oral NAC on the TS-induced hyperplasia of epithelial secretory cells is summarized in tables 1 and 2. In none of the experiments did NAC-treatment significantly effect the secretory cell numbers, either in the axial bronchus (table 2) or at the other bronchial...
levels (table 1). The secretory cell hyperplasia in NAC-treated animals was again dependent on the proliferation of acidic glycoprotein containing cells (only about 1% of the secretory cells stained only for neutral glycoproteins). In addition, oral NAC potentiated (p<0.001) the epithelial thickening induced by TS (table 1).

Treatment of nonsmoking rats with oral NAC alone did not affect the secretory cell number (table 2).

Respiratory tract fluid and its albumin content

In the control group of experiment 5 the median volume of RTF collected was 0.175 μl·min⁻¹·kg⁻¹ body weight (note absolute values are given for comparative purposes only). The corresponding figures for the TS and TS+NAC groups were 0.20 and 0.25 μl·min⁻¹·kg⁻¹ body weight, respectively (fig. 2), 14% (ns) and 43% (p<0.01) higher than in the control group. In experiment 6 the median volumes were control 0.163, TS 0.179 and TS+NAC 0.199 μl·min⁻¹·kg⁻¹ body weight (fig. 2). The two latter volumes were about 10% (p=0.07) and 22% (p<0.01) higher than the volume in the control group.

The median level for albumin output into the RTF was 50 and 39 μg·min⁻¹·kg⁻¹ body weight in the control groups of experiments 5 and 6 (fig. 3). TS enhanced this content in experiment 5 (40%, ns) but not in experiment 6 (-1%, ns). The albumin content was consistently enhanced in the TS+NAC group (by 44% and 41%, ns and p<0.05) in both experiments.

In a separate experiment, oral treatment with NAC of rats not challenged with the volume of RTF by about 50% (0.25 to 0.38 μg·min⁻¹·kg⁻¹, p<0.001), (fig. 2) and the albumin content by 100%, (73 to 145 μg·min⁻¹·kg⁻¹, p<0.01), (fig. 3).

Discussion

Orally administered NAC has been shown to be clinically effective in reducing exacerbation frequency in chronic bronchitis. To date, there is relatively little evidence to indicate how NAC may produce this therapeutic effect. NAC is known to be a mucolytic agent, yet after oral administration (600 mg·day⁻¹) no intact NAC has been found in the human airways [11, 12] and therefore NAC cannot have a direct mucolytic effect. Local administration of NAC at 1–5% concentrations has been found to be toxic to human bronchial cilia [13], and mucociliary transport rates in man have been shown to be unaffected [14] and increased [15, 16] by oral NAC at 600 mg·day⁻¹. It has recently been proposed that the protection achieved in bronchitis by oral NAC may be related to the action of the substance to inhibit TS-induced increase in secretory cell numbers, when given prophylactically to rats [17]. In the present study it was hoped to confirm the earlier reported [17] prophylactic effect of oral NAC on TS-induced increase in epithelial secretory cells in another model of TS-provocation.

In the present study the rats were exposed to puffs of TS alternating with short periods of fresh air. The TS was produced near to the noses of the rats. The provocation system used by ROGERS and JEFFERY [17] was based on a daily 4 h continuous smoke exposure, whereas the rats were sitting in their cages at some distance from the smoking machine; the composition of TS may then have differed in having a somewhat greater proportion of smaller particles (less prone to sedimentation) and of more long-lived oxidant radicals. The model with "nose provocation" via the nose may better simulate human active smoking regarding TS challenge of large airways. The experimental conditions of ROGERS and JEFFERY [17] may in other respects better mimic smoke challenge of small airways and long-term passive smoking. Both modes of provocation induced thickening of the tracheal epithelium and secretory cell hyperplasia in central airways. However, only the indirect provocation model [17] provoked secretory cell hyperplasia in the distal airways, which hyperplasia was found to be especially sensitive to the prophylactic action of NAC. However, the total lack of effect of NAC in the present test on the epithelial thickening of trachea and the epithelial secretory cell hyperplasia of the large airways show that prophylactic activity of NAC is not a general phenomenon. In neither model was neutrophilia found to be a feature in the tissues studied. Further studies are required in both models to better understand under what conditions oral NAC can protect against secretory cell hyperplasia.

The clinical definition of chronic bronchitis involves an increase in sputum volume. In the present rat model the volume of RTF increased by about 10% (of borderline significance) in the TS-group. It is not clear whether this slight effect depends on enhanced secretion from secretory cells or from enhanced transudation. In addition TS had a variable effect on albumin in the RTF giving an increase (ns) in one of the two experiments. It would be interesting to see if collection of RTF immediately after TS, as opposed to 24 h after, would have resulted in larger changes to volume and albumin concentration. In other experiments more clear-cut results have been demonstrated. JEFFERY et al. [9] have demonstrated in tracheal preparations that TS enhances the secretion of glycoproteins of mucous origin. TS has been shown to have an acute effect on airway epithelial junctions, allowing the passage of the tracer molecule horseradish peroxidase at all airway levels [18]. In humans, three days of cigarette smoking has been shown to acutely increase epithelial permeability to ⁹⁹Tc-diethylenetriamine penta-acetate (⁹⁹Tc-DTPA) [19].

When NAC was given to the rats it significantly increased both RTF volume and albumin content, the outputs from the TS+NAC group always being higher than the corresponding TS only group. The same results were also obtained in rats not challenged with TS. These results may be explained if NAC were causing an increase in RTF volume and albumin output via a permeability oedema type mechanism. Active transport of albumin into the airway lumen of ferrets has been...
It is possible that NAC could be stimulating the active transport of albumin into the airway lumen and that the increases in volume and albumin concentration were not related. It seems unlikely that NAC is increasing RTF volume by the stimulation of goblet cell secretion as no cell hyperplasia, which might then be expected, is seen in response to NAC-treatment alone.

It is interesting to note that Eklund et al. [21] found no change in albumin concentration in the bronchial lumen of patients taking 200 mg NAC t.i.d. for 5 weeks, although a significant increase in plasma albumin was noted: this apparent discrepancy may, however, be due to the fact that the NAC dose used in these experiments was about 100 times that of the routine human dose.

Whilst it cannot be concluded from these experiments that NAC has its mechanism of action in bronchitis through an inhibition of TS-induced secretory cell hyperplasia, a mechanism of action may be suggested by its capacity to increase RTF volume. In clinical trials a relative increase in sputum volume is frequently seen in humans but not always reported to occur with oral NAC [22-24]. It is possible, for example, that in bronchitics, a further NAC-induced increase in the volume of fluid within the airways may, by dilution of existing fluid, improve the rheological characteristics of the airways fluid and have a positive beneficial effect, perhaps by reducing duration of bacterial residency.

Additional studies in both rats and man are necessary to further quantify any oral NAC-induced changes to respiratory tract fluid and, if such changes are present, to determine how they may be beneficial in reducing the exacerbation frequency of bronchitics.

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References


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respiratoire en provenance des voies respiratoires inférieures de rats "bronchitiques". Les rats ont été exposés, soit à la fumée de tabac (TS), soit à TS et NAC, soit à NAC seule. TS a provoqué une augmentation significative du nombre de cellules sécrétories épithéliales, qui n'était pas diminuée par l'administration concomitante de NAC. NAC seule n'a aucun effet sur le nombre de cellules. TS a augmenté le volume du liquide du tractus respiratoire et le contenu en albumine de manière discale et non significative, tandis que TS et NAC ont augmenté le volume et le contenu en albumine de façon plus importante et significative. NAC seule s'est avérée capable d'augmenter significativement le volume de liquide et le contenu en albumine.