Oral N-Acetylcysteine Attenuates Elastase-Induced Pulmonary Emphysema in Rats*

Maria L. Rubio, PhD; M. Carmen Martin-Mosquero, MS; Mercedes Ortega, MS; German Peces-Barba, PhD; and Nicolás González-Mangado, PhD

Study objective: To study the effect of the antioxidant N-acetylcysteine (NAC) in the development of elastase-induced emphysema in rats.

Materials and methods: Wistar rats (n = 72) were orotracheally instilled with 75 IU elastase or saline solution. Eighteen rats from each group received the antioxidant NAC from 2 days before induction of the lesion until they were killed 2, 8, and 28 days after instillation. The effects of treatment were assessed by measuring collagen content for the left lung, a histopathology evaluation (ie, mean alveolar internal surface area (AIA) and mean linear intercept measurement), and lung function.

Results: Twenty-eight days after elastase instillation, rats treated with NAC showed significant attenuation of the lesion in comparison with rats treated only with elastase, including the following: normalization of mean (± SEM) collagen content (1.23 ± 0.09 vs 1.51 ± 0.10 mg per left lung, respectively; p < 0.05); partial inhibition of mean AIA (14,860 ± 1,135 vs 19,622 ± 1,294 μm², respectively; p < 0.05) and mean linear intercept (108.8 ± 3.7 vs 123.0 ± 4.2 μm, respectively; p < 0.05); and increases and improvement in expiratory flows (27.8 ± 1.2 vs 23.4 ± 1.3 mL/s, respectively; p < 0.05). NAC was not able to avoid the compliance increase in the elastase-plus-NAC group.

Conclusion: Consistent with the results of anatomic, pathologic, and functional studies, NAC is able to attenuate the lesions induced by elastase in rats, which is in accordance with previous data supporting the idea that oxidant injury could contribute to the development of elastase-induced emphysema.

Key words: elastase; emphysema; lung function; N-acetylcysteine; rats

Abbreviations: AIA = alveolar internal surface area; CL = lung compliance; F75 = expiratory flow at 75% of FVC; HYP = hydroxyproline; IC = inspiratory capacity; Lm = mean linear intersection; Lmc = computerized mean linear intersection; NAC = N-acetylcysteine; PMN = polymorphonuclear cell

Intratracheal elastase administration induces a lesion that resembles human panacinar emphysema.1 In hamsters, elastase initially provokes a severe decrease in the elastin content of the lung that is gradually recovered while the emphysema worsens.2 There also appears to be an increase in collagen synthesis during the development of emphysema.3 These changes in connective tissue after elastase administration, observed not only biochemically but also by electron microscopy,3 reflect the repair reaction of the lung after elastase-induced injury.

Functionally, the lesion produces an increase of inspiratory capacity (IC) and lung distensibility, and a decrease of expiratory flows. Morphologically, elastase provokes a disruption of the alveolar walls that leads to the enlargement of the airspace regularly distributed throughout all the parenchyma, which is reflected in an increase of the mean linear intercept.4

Furthermore, intratracheal elastase induces an early inflammatory response with neutrophils and macrophages,2 which is still present 1 month later.3 These cells could be the source of proteases and oxidants that can contribute to the destruction of lung connective tissue as well as of inflammatory mediators that exacerbate elastase-induced emphysema.5

*From the Laboratorio Neumología Experimental, Servicio de Neumología, Fundación Jiménez Díaz, Universidad Autónoma, Madrid, Spain. Supported in part by Zamboń SA and Red Respira (grant RTIC C03/11,FIS,ISCHII).

Manuscript received March 25, 2003; revision accepted September 1, 2003.

Reproduction of this article is prohibited without written permission from the American College of Chest Physicians (e-mail: permissions@chestnet.org).

Correspondence to: Nicolás González Mangado, MD, PhD, Servicio de Neumología, Fundación Jiménez Díaz Acda/ Reyes Católicos, 2 28040-Madrid, Spain; e-mail: ngonzalez@fjd.es

1500 Laboratory and Animal Investigations
N-acetylcysteine (NAC) is a precursor of glutathione molecules and has oxygen radical-scavenging properties. The effectiveness of NAC administration in animal models of lung fibrosis as well as in patients with idiopathic pulmonary fibrosis has been reported. In this respect, it has been demonstrated that NAC is able to attenuate cellular infiltration and collagen deposition in a model of bleomycin-induced lung fibrosis. When administered together with steroids, it improves the lung function index in patients with idiopathic pulmonary fibrosis. It also has been reported that NAC has an anti-inflammatory role because of its capacity to regulate the production of some inflammatory mediators in fibrosis induced in vitro and in vivo. To our knowledge, there are no studies about the effect of NAC administration on the evolution of induced pulmonary emphysema. The aim of this study was to investigate whether oral administration of antioxidant/anti-inflammatory NAC has any effect on the repair, lung function, and morphometry.

MATERIALS AND METHODS

Materials and Subjects

The Animal Research Committee of the center approved all animal experimentation. Male Wistar rats (weight range, 180 to 200 g) were classified into the following four groups: a control group (n = 18) orotracheally instilled with saline solution; an elastase group (n = 18) orotracheally instilled with 75 IU porcine pancreatic elastase (Roche Diagnostics; Basel, Switzerland); a control group treated with NAC (n = 18); and an elastase group treated with NAC (n = 18). After instillation, the rats were returned to their cages. The rats that were to receive NAC (Laboratorios Zambo SA; Barcelona, Spain) were given 200 mg NAC per rat per day mixed with powdered food divided into two doses (at 9:00 am and at 6:00 pm) beginning 2 days before the instillation until the study day. Food was provided ad libitum, checking that there was as little food as possible remaining between dosages in the groups receiving NAC. The dosage, which was equivalent to the human dosage in terms of body surface, was the same that proved to be effective ameliorating cigarette smoke-induced lung lesions in rats when used previously. Rats were killed 2, 8, and 28 days after instillation.

Lung Function Tests

The functional study was performed in a breathing assembly for small animals, as described elsewhere. The rats were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally), tracheotomized, put into a plethysmograph, and fitted to a cannula that allowed communication with the breathing assembly. As soon as the rat was connected to the breathing equipment, it was paralyzed with 0.2 mg pancuronium bromide and then artificially ventilated. Changes in IC, lung compliance (CL), and expiratory flow at 75% of FVC (P75) were assessed.

Statistical Analysis

All data are expressed as the mean ± SEM. Comparisons were made with analysis of variance. Multiple range tests (least significance differences method) were used for the analysis of differences among means. Pearson correlations also were calculated.

RESULTS

Collagen Content

The sequential results of collagen changes over time are represented in Figure 1. Collagen measured by HYP was significantly higher in elastase-treated rats at 8 days, with the difference increasing with respect to the control group after 28 days. Treatment with NAC significantly hindered the increase of HYP from the first days after instillation, which was significantly lower than that in the elastase group on the 28th day (1.23 ± 0.09 vs 1.51 ± 0.1 mg, respectively) and was not significantly different from that of the control group at any point in time.

Morphometry

In terms of airspace enlargement, the lesion was still not evident on or before the 8th day. For this reason, we have focused on Lm and area values after 28 days (Table 1). Rats treated with elastase-pre-
sented significantly increased AIA compared with that of the control group. As shown in Figure 2, the elastase-plus-NAC group showed significantly lower percentages of Lmc and AIA than the elastase group. Figure 3 shows histopathologic images of lung sections from the control, elastase, and elastase-plus-NAC groups obtained 28 days after induction of the lesion. The Lm followed the same tendency and correlated highly with Lmc ($r = 0.96; p < 0.05$).

The correlation between AIA and HYP also was calculated. As can be seen in Figure 4, 28 days after elastase administration, a significant positive correlation was found between AIA increase and HYP level in the elastase and control groups ($r = 0.46; p < 0.05$), but not in the elastase-plus-NAC group.

**Functional Study**

The course of function deterioration over time in the control, control-plus-NAC, elastase, and elastase-plus-NAC groups for IC, CL, and F75 percentages are represented in Figures 4 and 5. IC was significantly lower in the first days after elastase administration, but was not different from the saline control group after 28 days (Fig 5, left, A). CL (Fig 5, right, B) was not different from the control group in the first days after elastase administration, but was significantly higher in both elastase groups on the 28th day. However, on the 28th day the decline was significantly lower in the elastase-plus-NAC group compared with the elastase group. Correlations between functional and morphometric parameters after 28 days were calculated for the elastase and elastase-plus-NAC groups. As shown in Table 2, AIA correlated positively with CL and negatively with F75 in both elastase groups. In the elastase-plus-NAC group, the correlation with CL was higher and that with F75 was lower than those obtained in the elastase group, in absolute values. Lmc followed the same tendency.

**Discussion**

Treatment with oral NAC partially attenuates lung emphysema induced by elastase in rats. Improv-
ment is observed in the amelioration of airspace enlargements, the partial recovery of expiratory flows, and the normalization of lung collagen content.

We found that after elastase administration there was an evident increase of collagen from the 8th day. The higher collagen content in elastase-treated animals was initially found by Kuhn et al in hamsters, and later this event was described with detail using scanning electron microscopy in rats. The alveolar structures of the lung seem to initiate repair responses after structural injury. In this way, elastin and collagen gene expression is up-regulated after elastase administration. There is some evidence suggesting that inadequate repair contributes to the worsening of emphysema. For example, starvation, which generally inhibits anabolic responses, can exacerbate the development of elastase-induced emphysema in animals. In addition, the inhibition of elastin synthesis by β-aminopropionitrile, a lathyrogen that inhibits elastin maturation, worsens emphysema in elastase-exposed animals. Our data demonstrate that the repair reaction started after elastase administration does not, however, efficiently restore tissue integrity. Morphometric determinations after 28 days showed that elastase provoked an enlargement of alveolar spaces reflected in an increase of AIA of 280% with respect to the control group, which is compatible with the increase in Lmc. Moreover, we found that the higher amount of HYP in the elastase group correlated positively with airspace enlargement, quantified in terms of AIA (r = 0.46; p < 0.05).

Figure 3. Light microscopic photomicrograph of lung parenchyma 28 days after the induction of the lesion. Top left, A: control group (Lm, 89 μm). Top right, B: elastase group (Lm, 175 μm). Bottom, C: elastase-plus-NAC (Lm, 118.5 μm) [hematoxylin-eosin, original ×100]. The enlargement of airspaces in the elastase group (top right, B) is partially inhibited by the administration of NAC (bottom, C).
Interestingly, the daily administration of NAC for 28 days after elastase instillation significantly reduced lung collagen content compared to the elastase group. Morphometrically, the emphysema was less severe, with both AIA and Lmc being significantly lower than that in the elastase group (reduction of 25% for AIA and 12% for Lmc compared to elastase group). Functionally, expiratory flow was significantly improved after NAC treatment, although values still remained lower than those in the control group. However, CL was not influenced by NAC and was equally higher in both elastase groups, suggesting that NAC does not interfere in the elastolytic process. Nevertheless, the correlation between CL and AIA (Table 2) not only persisted in the elastase-plus-NAC group but was even higher than that for the elastase group. This could mean that in the elastase-plus-NAC group the increased distensibility is mainly explained by alveolar enlargement. In contrast, in the elastase group several other factors could be involved that may interfere in the relationship between the increase in AIA and distensibility. Taking into account the lack of change in CL, the improvement of expiratory flow in the elastase-plus-NAC group only could be explained by an increase in airway conductance in rats treated with NAC. In a similar study, retinoic acid was administered daily for 2 weeks, 25 days after elastase instillation. However, despite notable lung morphology results, retinoic acid was not able to reverse either increased CL or expiratory flows and anatomic-functional correlations were not stated.

Is difficult to believe that NAC prevents the direct enzymatic effect of elastase. It must be acting at a different level. Previous studies have demonstrated

![Figure 5](Image)

**Figure 5.** IC (left, A) and compliance (right, B) of the control, control-plus-NAC, elastase, and elastase-plus-NAC groups 2, 8, and 28 days after elastase instillation. * = significantly different from the control group (p < 0.05).

![Figure 6](Image)

**Figure 6.** F75 reduction at 2, 8, and 28 days after elastase instillation for the control-plus-NAC, elastase, and elastase-plus-NAC groups, expressed as a percentage of the control value. F75 was significantly decreased in the elastase and elastase-plus-NAC groups compared to the control group at 2, 8, and 28 days (p < 0.05). * = significantly different from the control group (p < 0.05); † = F75 decline was significantly smaller in the elastase-plus-NAC group than in the elastase group at 28 days (p < 0.05).

### Table 2—Pearson Correlations Between Morphometry and Lung Function Parameters at 28 Days

<table>
<thead>
<tr>
<th>Variables</th>
<th>Elastase Group</th>
<th>Elastase + NAC Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AIA Lmc</td>
<td>AIA Lmc</td>
</tr>
<tr>
<td>IC</td>
<td>NS</td>
<td>+0.45</td>
</tr>
<tr>
<td>CL</td>
<td>+0.55</td>
<td>+0.72</td>
</tr>
<tr>
<td>F75</td>
<td>-0.70</td>
<td>-0.48</td>
</tr>
</tbody>
</table>

*Correlations are significant at p < 0.05. NS = not significant.
that 2 h after elastase administration its activity is markedly reduced\textsuperscript{22} and is cleared from the lung within 24 h.\textsuperscript{23} However, the development of emphysema is gradual over a span of at least 2 months,\textsuperscript{22} suggesting that endogenous mechanisms must be involved.

It has been reported that during the first 48 to 72 h after elastase administration there is a moderate acute inflammatory response with neutrophils and macrophages,\textsuperscript{2} and that 4 weeks later there is still a markedly elevated total cell count.\textsuperscript{3} Activated inflammatory cells can release oxygen radicals and proteases that can directly damage components of the lung matrix. Moreover, it has been demonstrated\textsuperscript{24} that several proteases induce a direct rise in reactive oxygen species in bronchial epithelial cells and fibroblasts. On the other hand, Leff et al\textsuperscript{25} described a decrease in neutrophil influx into lung lavage fluid after NAC administration in an animal model treated with interleukin-1\textalpha.

In addition, genes for many inflammatory mediators, some of them involved in the recruitment of inflammatory cells and, therefore, in the development of emphysema,\textsuperscript{5} are regulated by transcription factors such as nuclear factor \( k \)B. Nuclear factor \( k \)B activation can be induced by oxidants,\textsuperscript{26} and NAC previously has been demonstrated to be efficient in decreasing this activation in \textit{vivo}.\textsuperscript{27} In the elastase-induced emphysema model, NAC could be acting at this level by hindering the overexpression of genes for inflammatory cytokines.

Thus, lesions induced in rats by elastase instillation seem to be not only the consequence of the initial enzymatic damage but, more probably, the result of several secondary stimuli that lead to the worsening of the disease over time. Lucey et al\textsuperscript{5} recently stated that tumor necrosis factor-\( \alpha \) and interleukin-1\( \beta \) account for about 50\% of the emphysema that develops after elastase treatment, pointing out that these proinflammatory mediators (polymorphonuclear cell [PMN] chemoattractants) are taking part in the development of the lesion. But actually, there is no evidence showing that the elastase-induced emphysema is accompanied by an increase in the number of PMN cells. According to these data, Cantor et al\textsuperscript{28} found that 1 week after the induction of the lesion with elastase the number of PMNs was normal. In our experiment, it is not possible to know whether NAC treatment completely abolishes the oxidative stress occurring after elastase, but we think that oxidants can explain at least part of the emphysematous lesion, taking into account the possible anti-inflammatory effect of NAC throughout the diminution of PMN influx into the lung.

The effectiveness of NAC in attenuating bleomycin-induced lung fibrosis is unquestionable,\textsuperscript{8—10} although the mechanism by which it limits fibrosis is also unclear. Bleomycin damage consists of the following two phases: an early inflammatory phase, characterized by cellular infiltration; and a late fibrotic phase. Reactive oxygen species and proteases generated from infiltrated cells are considered to injure the lung tissue, and excessive fibrosis occurs as a reparative process. These two phases also seem to be present in emphysema development, the second chronic severe reparative phase establishing the different pathophysiologies of both diseases.

In patients with COPD, the oxidant burden is also increased. Cigarette smoke, the major etiologic factor in COPD, is a rich source of oxidant molecules. Oxidative stress is also a critical feature in the pathogenesis of COPD since it results in the inactivation of antiproteinas, airspace epithelial injury, mucus hypersecretion, increased influx of neutrophils into the lungs, transcription factor activation, and gene expression of proinflammatory mediators. Antioxidants, therefore, should not only protect against the direct injurious effects of oxidants, but may also fundamentally alter the inflammatory events that have a central role in the pathogenesis of COPD.\textsuperscript{29}

Fibrosis, emphysema, and COPD seem to have the oxidant/antioxidant imbalance in common. The site and specific characteristics of the inflammatory responses may differ in each of these diseases.

In conclusion, oxidant/antioxidant imbalance seems to contribute to the development of elastase-induced emphysema, and treatment with antioxidant NAC could attenuate or slow down the process.

\textbf{References}

5 Lucey EC, Keane J, Kuang P-P, et al. Severity of elastase-induced emphysema is decreased in tumor necrosis factor-\( \alpha \) and interleukin-1\( \beta \) receptor-deficient mice. Lab Invest 2002; 82:79–85