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Attenuation by oral *N*-acetylcysteine of bleomycin-induced lung injury in rats

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ABSTRACT: Antioxidant therapy may be useful in diseases with impaired oxidant-antioxidant balance such as pulmonary fibrosis. This study examines the effect of *N*-acetylcysteine (NAC) on bleomycin-induced lung fibrosis in rats.

NAC (3 mmol·kg^{-1}; oral) was given daily from 1 week prior to a single intratracheal instillation of bleomycin (2.5 U·kg^{-1}) or saline, until 14 days postinstillation.

NAC partially decreased the augmented collagen deposition in bleomycin-exposed rats (hydroxyproline content was 4,354±386 and 3,416±326 µg·lung^{-1} in vehicle-treated and NAC-treated rats, respectively; $p < 0.05$). The histological assessment using a semiquantitative score showed less collagen deposition and inflammatory cells in NAC-treated rats compared to those receiving bleomycin alone. NAC failed to inhibit the bleomycin-induced increases in lung wet weight and in cell counts and protein levels of bronchoalveolar lavage fluid, but significantly increased total glutathione and taurine levels in bronchoalveolar lavage fluid.

These results indicate that oral *N*-acetylcysteine improves the pulmonary antioxidant protection and may be useful in reducing lung damage produced by bleomycin.

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Idiopathic pulmonary fibrosis is a chronic inflammatory interstitial lung disease of potential fatal prognosis and poor response to available medical therapy [1]. It has been hypothesized that activated inflammatory cells which accumulate in the lower airways release increased amounts of reactive oxygen species (ROS) which, combined with a deficiency in glutathione, the major component of the lung antioxidant defense system, produces lung injury and fibrosis [2]. The antioxidant *N*-acetylcysteine (NAC) has shown beneficial effects in diseases in which ROS appear involved [3]. In short-term studies, NAC improved the antioxidant screen of the lung by elevating glutathione levels in patients with pulmonary fibrosis accompanied by better pulmonary function and low incidence of adverse effects [4–6].

One of the clinically important causative agents in pulmonary fibrosis is bleomycin, an antineoplastic drug widely used in experimental models of pulmonary fibrosis resembling human disease [7], and used to assess potential therapeutic agents including NAC [8–14]. NAC exerts direct antioxidant properties as a free radical scavenger and, as an L-cysteine prodrug, increases reduced glutathione in airway cells under oxidant stress [3, 15]. In addition, benefit could be afforded by increased synthesis of taurine following NAC administration [16], since taurine protects against oxidant damage in the lung [17].

The aim of the present study was to examine the effects of orally administered NAC in a rat model of lung injury produced by endotracheal bleomycin. Lung damage was assessed by a semiquantitative histological score, and lung hydroxyproline was measured as a marker of collagen deposition. Cell counts, protein and glutathione levels in bronchoalveolar lavage fluid (BALF) were also determined. In addition, the changes in taurine levels in BALF, plasma and granulocytes were measured following NAC administration.

Materials and methods

Drug sources

Bleomycin sulphate was from Almirall-Prodesfarma (Barcelona, Spain) and *N*-acetyl-L-cysteine from Zambon (Bresso, Italy). Other chemicals and reagents were from standard commercial sources.

Animal model

Pathogen-free, male Sprague-Dawley rats, weighing 225–250 g at the start of experiments, were obtained from B&K Universal G.J.S.L. (Barcelona, Spain). Rats were fed rodent chow (A04; Panlab, Barcelona, Spain) and were maintained in a 12-h light-dark cycle. This study conformed to European Community (Directive 86/609/EEC) and Spanish guidelines for the use of experimental animals and it was approved by the institutional committee of animal care and research.

To produce pulmonary fibrosis, animals received endotracheally, by the transoral route, a single sublethal dose of bleomycin (2.5 $U \cdot kg^{-1}$ dissolved in 0.25 mL of 0.9% NaCl). Control animals were subjected to the same protocol but received the same volume of intratracheal saline instead of bleomycin. Tracheal instillation was carried out under halothane anaesthesia. Rats were weighed every 3 days. Fourteen days after endotracheal bleomycin or saline, the animals were killed by a lethal injection of sodium pentobarbital followed by exsanguination from abdominal aorta. Bronchoalveolar lavage was performed and lungs were weighed and processed separately for biochemical and histological studies as indicated below. The 14^{th} day after bleomycin was selected as the approximate time for maximal rate of collagen synthesis [13].

Experimental groups

The animals were randomly divided into four groups (group A: vehicle + vehicle; group B: NAC + vehicle; group C: vehicle + bleomycin; and group D: NAC + bleomycin). Treatments (vehicle or NAC) were administered orally by gavage on a daily basis (at 9:00 h) from 7 days prior to the intratracheal instillation of bleomycin or saline up to the conclusion of the experiments 14 days postinstillation. Oral NAC was selected as the usual way of administration in the clinical setting [4, 6], and also because this route had not been previously examined in this rat model. The oral dose of NAC was 3 $mmol \cdot kg^{-1}$ per day, given as a single dose in a final volume of 1 mL of distilled water as vehicle. The dose level and schedule were based on previous studies [11–13].

Additional experiments were carried out to examine the influence of dose level of NAC and its time of administration as follows: 1) a lower dose of oral NAC (0.3 $mmol \cdot kg^{-1}$) was administered daily from 7 days prebleomycin to 14 days post-bleomycin; and 2) oral NAC (3 $mmol \cdot kg^{-1} \cdot day^{-1}$) was given from one day before or 7 days after bleomycin challenge to 14 days postbleomycin. Control groups received vehicle + vehicle and vehicle + bleomcyin. In these additional experiments, hydroxyproline quantification was used to assess pulmonary fibrosis.

Bronchoalveolar lavage

BALF was obtained by washing the right lung four times with 4 mL aliquots of saline through a tracheal cannula. Cell suspensions were concentrated by low speed centrifugation, and the cell pellet resuspended. Total cell counts were made in a haemocytometer. Differential cell counts were determined from cytospin preparations by counting 300 cells stained with May-Grünwald-Giemsa. Total protein content in BALF supernantants was measured by adding 10 μL of each sample to 90 μL of 0.9% NaCl together with 1 mL of Coomassie blue reagent (Bio-Rad; Munich, Germany). Absorbances were determined at 595 nm using a spectrophotometer.

Histological assessment

For histological studies, the left lung was first perfused by its main bronchus with a fixative solution (10% neutral-buffered formalin) at a pressure of 25 cmH_2O, immersed in the fixative for 12–24 h, and blocks taken. Tissue blocks were placed in formalin, dehydrated in a graded series of ethanols, embedded in paraffin, cut into 4 μm-thick serial sections, and stained with haematoxylin-eosin and Masson's trichrome to identify inflammatory cells, connective tissue and collagen deposition. Histologic grading of lesions was performed by two experienced histopathologists using a blinded semiquantitative scoring system for extent and severity of inflammation and fibrosis in lung parenchyma, as previously outlined [18]. Briefly, three lung sections from each animal were systematically scanned using a ×10 objective and each successive field was scored using the following grading scheme: grade 0 for normal tissue, grades 1–4 for presence of pulmonary inflammation and fibrosis. The severity of lesions was graded as 1 (mild), 2 (moderate), 3 (severe) and 4 (severe inflammation accompanied by total distortion of structure). The extent of lesions was graded as 1 (<10% of the slide), 2 (10–40%), 3 (40–70%), and 4 (>70% of tissue affected). Fields predominantly occupied by portions of large bronchi or vessels were not counted. The pattern of distribution of the lesions was defined as multifocal and/or diffused, with or without affectation of the subpleural zone. Oedema was scored progressively as perivascular (grade 1), interstitial (grade 2), intra-alveolar (grade 3) and organized oedema (grade 4). Infiltration of inflammatory cells was graded 0–4 relating to their increasing presence in the interstitial, peribronchiolar and intra-alveolar spaces by counting each cell type in 10 random fields.

Biochemical studies

Lung hydroxyproline content was measured as outlined by WOESSNER [19]. Samples were homogenized and then hydrolyzed in 6 N HCl for 18 h at 110°C. The hydrolysate was then neutralized with 2.5 M NaOH. Aliquots (2 mL) were analysed for hydroxyproline content after the addition of 1 mL of chloramine T, 1 mL of perchloric acid, and 1 mL of dimethylaminobenzladehyde. Samples were read for absorbance at 550 nm in a spectrophotometer. Results are expressed as mg of hydroxyproline per lung.

Total glutathione was measured in aliquots of BALF using the glutathione reductase-5,5′-dithiobis-(2-nitrobenzoic acid) recycling assay described by TIETZE [20], and the results are expressed as glutathione equivalents (2GSH + GSSG) in $nmol \cdot mL^{-1}$.

Taurine levels were measured in BALF, blood plasma and peripheral polymorphonuclear leukocytes. These cells were selected as relevant to the bleomycin model [14], and influenced by taurine [21]. Taurine was measured by a fluorometric technique that uses a derivatization with o-phthaldialdehyde prior to high-performance liquid chromatography (HPLC) [22]. The limit of detection of this technique has been established at 5 pmol per analysis which is adequate for levels found in BALF, plasma and granulocytes of rodents [17, 21].

Statistical analysis of results

Data are expressed as mean ± SEM. Statistical analysis was carried out by analysis of variance (ANOVA) followed by appropriate *post hoc* tests including Bonferroni correction and unpaired t-test. Significance was accepted when $p < 0.05$.

Results

Body weight

All the animals survived for the 21-day duration of the study. The body weight of rats not exposed to bleomycin (groups A and B) increased with time without any significant difference between vehicle- (group A) and NAC-treated groups (fig. 1). The rats in group C (vehicle + bleomycin) failed to gain weight during the first week after bleomycin instillation; thereafter weight gain paralleled that observed in rats not exposed to bleomycin. A similar, but less marked trend was noticed for rats in group D (NAC + bleomycin; fig. 1).

Lung weight and hydroxyproline levels

Bleomycin produced a significant increase in lung weight (fig. 2a). In rats exposed to bleomycin and treated with NAC, there was a tendency for lower lung weights but statistical significance was not reached. The lung hydroxyproline levels, a marker of collagen deposition, were increased approximately two-fold in bleomycin-treated rats compared to rats

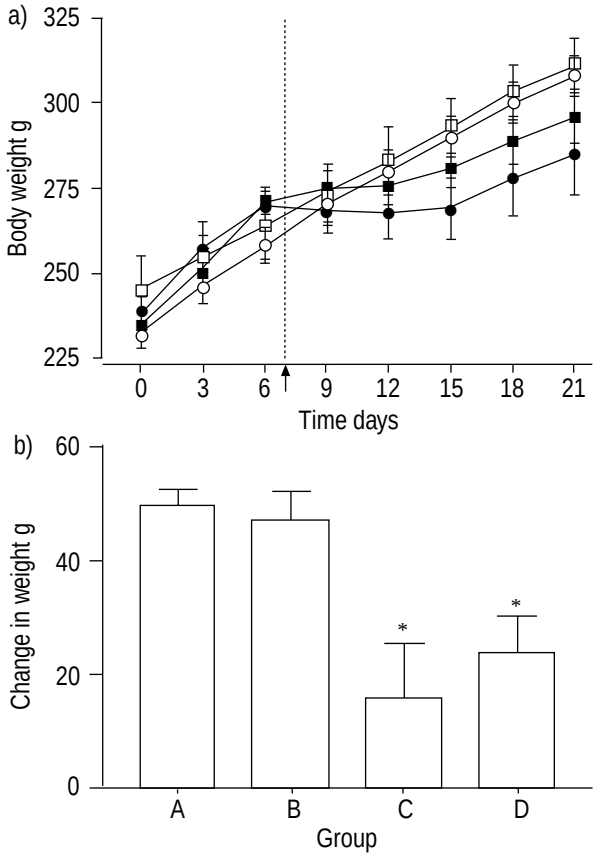

Fig. 1. – a) Time-course of the change in the body weight of the rats in the different experimental groups; ○: Group A (vehicle + vehicle); □: Group B (acetylcysteine (NAC) + vehicle); ●: Group C (vehicle + bleomycin); ■: Group D: NAC + bleomycin; ↑: bleomycin or vehicle administration. b) Comparison of the gain in body weight at day 21 from day 6 indicating that rats exposed to bleomycin experienced less weight gain compared to unexposed rats. Although rats treated with NAC and exposed to bleomycin showed a tendency for greater weight gain, the difference failed to reach significance. All data are presented as mean ± SEM of 10 (A), 6 (B) and 11 (C and D) animals. *: $p < 0.05$ *versus* A.

that received saline intratracheally. Treatment with NAC significantly reduced the hydroxyproline content, although it remained higher than levels in rats not exposed to bleomycin (fig. 2b).

Total and differential cell count and proteins in bronchoalveolar lavage fluid

Typically, total fluid recovery exceeded 80%, and the percentages of fluid recovered did not significantly differ among experimental groups. The pulmonary inflammation response after bleomycin administration, as reflected by the cells recovered in BALF, is shown in table 1. The total cell count was significantly increased in bleomycin-treated rats compared to in rats not exposed to bleomycin. The differential cell count showed that neutrophils were markedly augmented while the changes in the rest of the cell types failed to reach significance. NAC was without effect on total and differential cell numbers (table 1).

Protein in BALF was significantly increased in rats

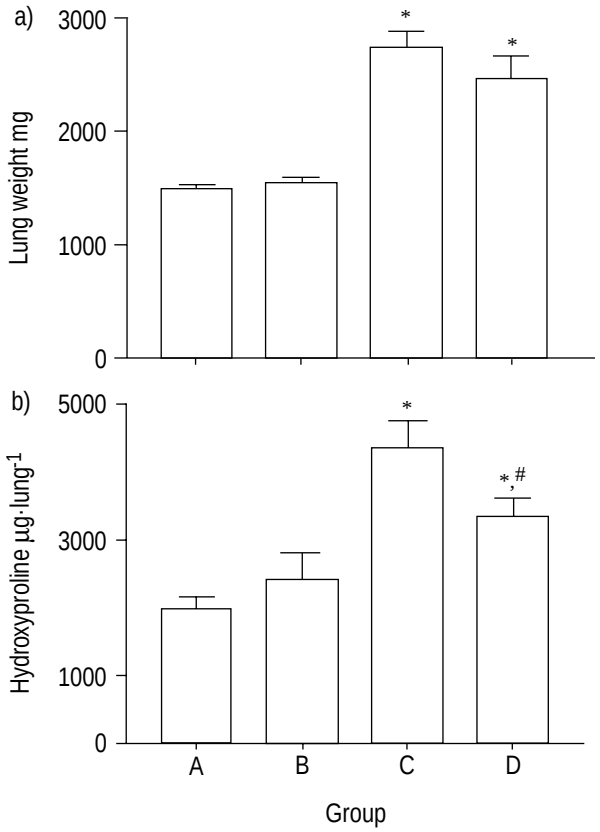

Fig. 2. – a) Lung weight and b) lung hydroxyproline levels in experimental groups (A, B, C, D) as indicated. Treatment with *N*-acetylcysteine (NAC) reduced the lung content of hydroxyproline. Data are mean±SEM of 10 (A), 6 (B) and 11 (C and D) animals; *: $p<0.05$ from A; #: $p<0.05$ *versus* C.

instilled with bleomycin. NAC-treated rats exposed to bleomycin showed a tendency to exhibit less protein content but the difference failed to reach significance compared to rats not exposed to bleomycin (table 1).

Histopathology

Masson trichrome and haematoxylin-eosin stained lung sections were examined by light microscopy to determine whether bleomycin-induced pulmonary fibrosis was decreased by treatment with NAC. Lungs from rats in groups A (vehicle+vehicle) and B (NAC+vehicle) were histologically normal (not shown). Lungs from rats in group C (vehicle+bleomycin) showed marked peribronchiolar and interstitial infiltration with inflammatory cells (predominantly mononuclear cells including macrophages and lymphocytes with fewer numbers of neutrophils and scattered eosinophils), extensive cellular thickening of interalveolar septa, interstitial oedema, increases in interstitial cells with a fibroblastic appearance and in interstitial collagen deposition detected by the trichrome stain, and association with focal cuboidal metaplasia of alveolar lining cells (fig. 3a and 3b). The pattern of distribution of lesions was multifocal (*i.e.* patchy areas of pulmonary fibrosis) in most cases, commonly involving the pleura.

Although multifocal parenchimal lesions were still present in lungs from rats of group D (NAC+blemocyin), the organized foci were less frequent and smaller than those seen in untreated animals, showed less oedema and collagen deposition, and less septal widening and clusters of inflammatory cells. (fig. 3c and 3d). A semiquantitative score of the severiry and extent of inflammation and fibrosis showed that most of the indices were reduced in group D compared to group C, yet significance was reached only for macrophages and the severity and extension of fibrosis (table 2).

Glutathione levels

Glutathione levels in BALF were not modified by NAC in control rats (not exposed to bleomycin). In the bleomycin-exposed animals not treated with NAC, there was a tendency to exhibit lower glutathione levels, and NAC treatment resulted in a significant increase of glutathione in BALF (fig. 4).

Taurine levels

Since NAC is a cysteine prodrug, this study explored the metabolic fate of cysteine by examining whether oral treatment with NAC increased the taurine levels in BALF, plasma and granulocytes. Figure 5a shows that taurine levels were increased in groups treated with NAC but not exposed to bleomycin. Bleomycin alone also increased taurine

Table 1. – The effects of bleomycin and *N*-acetylcysteine (NAC) on total and differential cell counts and proteins in bronchoalveolar lavage fluid

	Group A vehicle+vehicle	Group B NAC+vehicle	Group C vehicle+bleomycin	Group D NAC+bleomycin
Total cells $\times 10^6$ mL^{-1}	0.26±0.05	0.31±0.04	0.98±0.13*	1.01±0.19*
Neutrophils %	2.4±1.1	1.0±0.7	18.18±3.95*	21.18±6.93*
Eosinophils %	0.2±0.2	0.0±0.0	1.55±0.97	0.73±0.45
Lymphocytes %	10.6±4.2	11.8±1.3	6.64±1.94	7.09±2.16
Macrophages %	86.8±3.8	87.2±1.2	74.55±4.62	71.00±7.68
Proteins µg·mL	118±12	134±6	1047±87*	772±88*

Data are presented as mean±SEM of 10 (group A), 6 (group B), and 11 (groups C and D) experiments. *: $p<0.05$ *versus* group A. Differences between groups C and D failed to reach significance statistically.

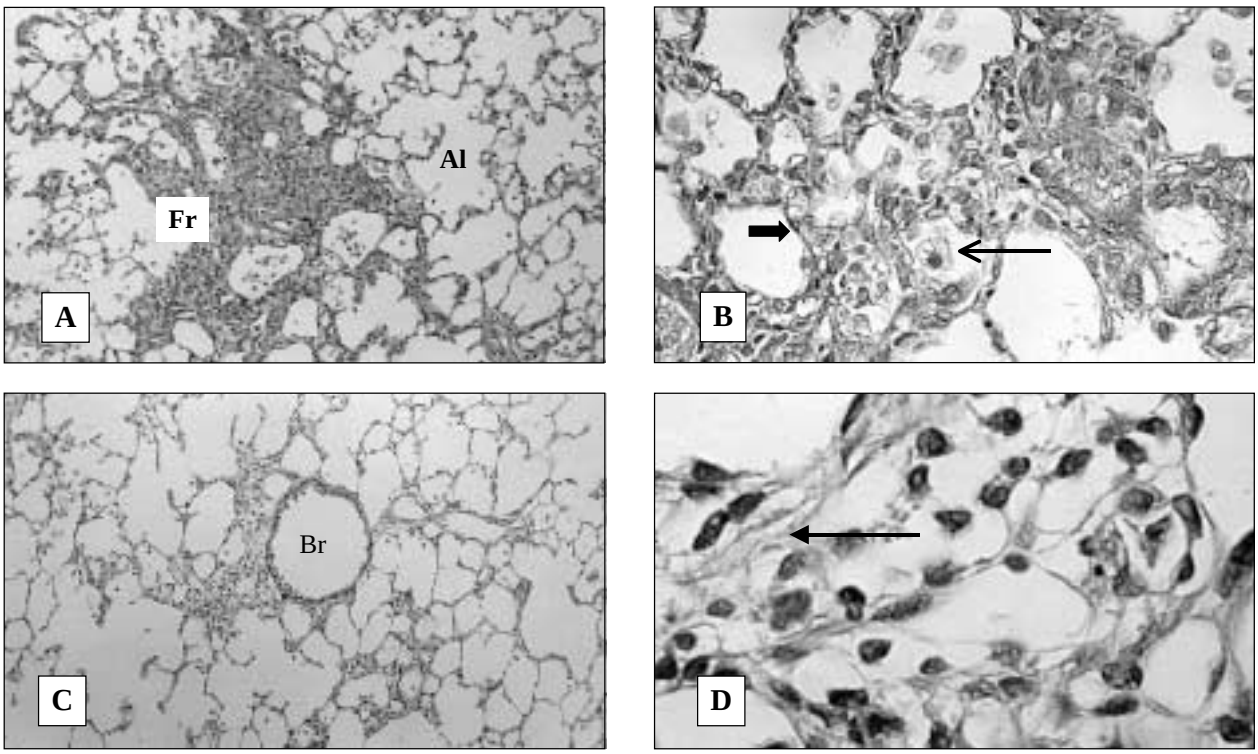

Fig. 3. – Representative photomicrographs of lung histopathology in groups C (vehicle + bleomycin; panels A and B) and D (*N*-acetylcysteine + bleomycin; panels C and D). Normal lungs observed for groups A (vehicle + vehicle) and B (*N*-acetylcysteine + vehicle) are not shown. Fourteen days after intratracheal bleomycin, a marked peribronchial interstitial infiltration with inflammatory cells, oedema and fibrosis were patent (A and B). These pulmonary lesions were markedly reduced in animals orally treated with *N*-acetylcysteine (C and D). Fr: fibrosis; Al: alveoli; Br: Bronchus; thick arrow in panel B indicates thickening of interalveolar septum and cuboidal metaplasia; thin arrow in panel B indicates a foamy intra-alveolar macrophage; arrow in panel D identifies interstitial collagen. Original magnification of $\times 10$ for A and C, and $\times 40$ for B and D.

levels in BALF, and NAC treatment produced a further increase of BALF taurine levels.

Although taurine levels in plasma were not significantly increased by treatment with NAC in rats not exposed to bleomycin, the plasma levels of taurine were augmented in rats receiving bleomycin, and treatment with NAC further enhanced the taurine concentration in plasma (fig. 5b). By contrast, the taurine levels measured in granulocytes did not show significant changes in rats irrespective of bleomycin exposure or treatment with NAC (fig. 5c).

Influence of dose level and time of administration of N-*acetylcysteine*

In these experiments, the lung hydroxyproline content in untreated rats exposed to bleomycin was $4{,}229 \pm 203$ $\mu g \cdot lung^{-1}$ ($n=6$, $p<0.05$ compared to a value of $2{,}298 \pm 154$ $\mu g \cdot lung^{-1}$ obtained in control rats not challenged with bleomycin, $n=6$). Rats receiving a lower dose of NAC (0.3 $mmol \cdot kg^{-1}$) from 7 days prebleomycin, showed no reduction in lung hydroxyproline ($4{,}090 \pm 189$ $\mu g \cdot lung^{-1}$; $n=6$, $p>0.05$ compared to vehicle + bleomycin). When NAC (3 $mmol \cdot kg^{-1}$) was given from one day prebleomycin, hydroxyproline values were decreased ($3{,}232 \pm 110$ $\mu g \cdot lung^{-1}$; $n=6$, $p<0.05$ compared to vehicle + bleomycin) but the same dose of NAC administered from 7 days postbleomycin instillation failed to reduce hydroxyproline levels ($4{,}301 \pm 244$ $\mu g \cdot lung^{-1}$; $n=6$, $p>0.05$ compared to vehicle + bleomycin).

Discussion

The results of the present study show that prior treatment with oral NAC was partially effective to reduce the lung damage produced by intratracheal instillation of bleomycin in rats. The observed benefical effect is associated with the diminished accumulation of collagen, assessed as lung hydroxyproline content, as well as with the improvement of pathologic grading. However, NAC failed to inhibit the bleomycin-induced increases in lung wet weight and in inflammatory cell counts and protein levels of BALF.

The potential of NAC to attenuate lung damage and subsequent fibrosis remains controversial in the literature. Thus, in the rat, intratracheal NAC (~ 200 $mg \cdot kg^{-1}$) abolished the lung injury subsequent to simultaneously administered bleomycin (~ 5 $U \cdot kg^{-1}$, intratracheal) [8] but intraperitoneal or subcutaneous NAC (~ 200 $mg \cdot kg^{-1} \cdot day^{-1}$) started before bleomycin (~ 5 $U \cdot kg^{-1}$, intratracheal), failed to ameliorate lung toxicity [9, 10]. In hamsters, NAC (200 $mg \cdot kg^{-1} \cdot day^{-1}$, intraperitoneal) did not reduce lung damage elicited by endotracheal bleomycin (7.5 $U \cdot kg^{-1}$) [12]. In mice, oral

Table 2. – Histological lesions scores of lungs from normal rats (group A: vehicle + vehicle) and lungs from rats receiving endotracheal bleomycin in the absence (group C) and presence (group D) of a treatment with oral *N*-acetylcysteine

	Inflammation								Fibrosis	
	Animals	Severity	Distribution[#]			Cells (scale 0 – 4)			Oedema	Severity	Extension
	n	(scale 0 – 4)	Multifocal	Diffuse	Subpleural	M	N	L	(scale 0 – 4)	(scale 0 – 4)	(scale 0 – 4)
Group A	5	0 ± 0								0 ± 0	0 ± 0
Group C	7	2.00 ± 0.29	86	43	71	2.86 ± 0.13	1.29 ± 0.17	1.29 ± 0.17	1.14 ± 0.24	2.29 ± 0.26	2.86 ± 0.13
Group D	11	1.64 ± 0.24	82	27	36	1.73 ± 0.19*	0.91 ± 0.21	1.00 ± 0.19	1.09 ± 0.16	1.09 ± 0.09*	1.91 ± 0.21*

Data are presented as mean ± SEM. M: macrophages; N: neutrophils; L: lymphocytes; [#]: the percentage of animals in which the mentioned pattern of distribution was observed.
*: $p < 0.05$ *versus* Group C.

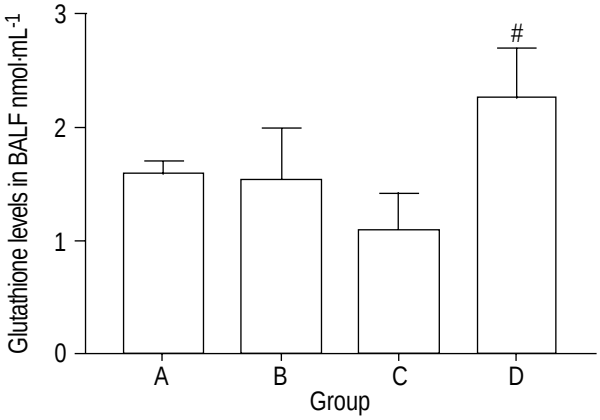

Fig. 4. – Glutathione levels in bronchoalveolar lavage fluid (BALF) in different experimental groups (A, B, C, D) as indicated. Data are presented as mean ± SEM of 6 (A), 5 (B), and 11 (C and D) animals; [#]: $p < 0.05$ *versus* C.

NAC (~400 mg·kg^{-1}·day^{-1}, before and after intratracheal bleomycin ~7 U·kg^{-1}) reduced lung collagen content, although lung wet weights and histopathology was not improved. [13]. Also in mice, NAC (400 mg·kg^{-1}) given as a single intraperitoneal dose prior to combined hyperbaric oxygen (445 kPa) and bleomycin (~7 U·kg^{-1}, endotracheal) was effective to protect against the lung damage that ensues these noxious stimuli [11]. Recently, aerosolized NAC was reported to attenuate lung fibrosis produced by systemic bleomycin in mice [14]. There are several possible explanations for these differing results. The observed effects will depend on the dose level of bleomycin, the timing between bleomycin and NAC administration, the animal species and strain, and even the criteria for detection of lung injury. The dose level of NAC shown to be effective in this study (~490 mg·kg^{-1}) is close to effective doses in mice [11, 13]. A lower dose (~50 mg·kg^{-1}) was also found to be ineffective, in keeping with other studies [23]. At the effective dose level, this beneficial effect of NAC was only partial, which is consistent with other recent reports [13, 14]. The effects of higher doses of NAC were not tested, but beneficial effects appear insurmountable in other studies [11]. This work used a dose of bleomycin (2.5 U·kg^{-1}) lower than that used by others [8 – 13]. This dose level causes no mortality in spite of producing pulmonary fibrosis consistently, and eases detection of pharmacological effects. Similar and lower intratracheal doses of bleomycin have recently been used for studies in this model [24].

The mechanism by which NAC limits fibrosis is unclear, but is likely to be *via* its ability to reduce damage to lung structures in the early stage of disease, since NAC administration from 7 days postbleomycin in the present study failed to influence the ensuing pulmonary fibrosis. This finding is consistent with a recent report showing that aerosolized NAC loses its effectiveness against pulmonary fibrosis when given after bleomycin administration [14]. Pulmonary injury produced by bleomycin probably involves generation of oxidant species by an iron-dependent mechanism [7]. Further damage is probably elicited by increased

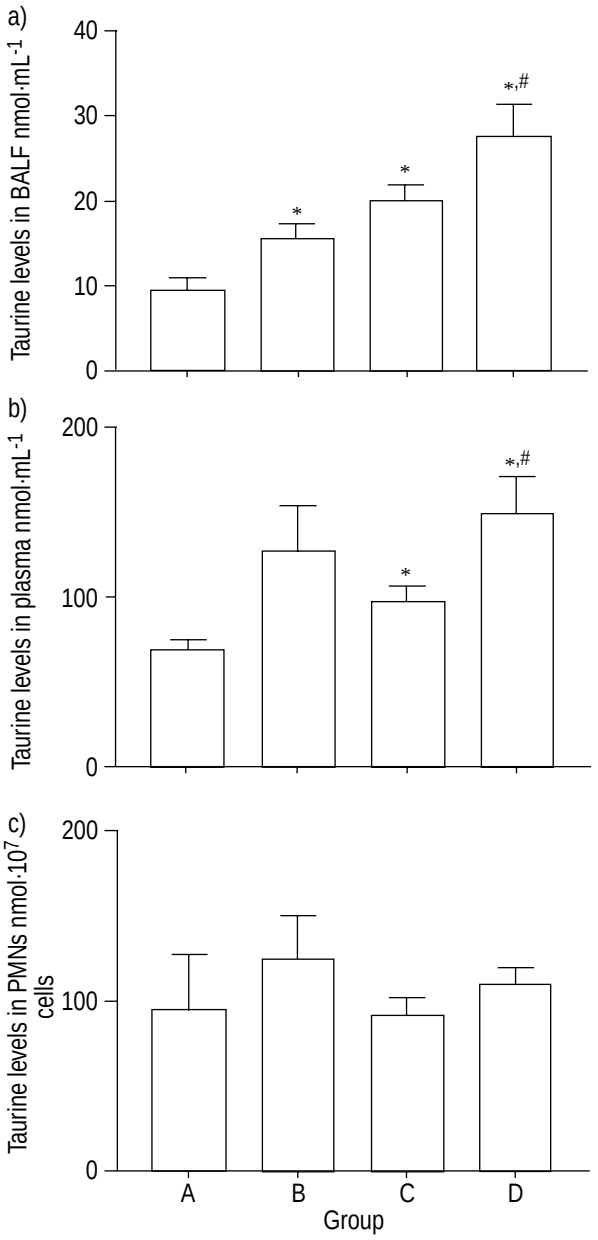

Fig. 5. – Taurine levels in a) bronchoalveolar lavage fluid (BALF), b) plasma and c) polymorphonuclear leukocytes (PMNs) in different experimental groups (A, B, C, D) as indicated. Data are presented as mean ± SEM of 5 (A), 6 (B), and 11 (C and D) animals; *: $p<0.05$ *versus* A; #: $p<0.05$ *versus* C.

amounts of ROS, produced by activated inflammatory cells which accumulate in the pulmonary lesions induced by bleomycin [2]. NAC may act directly as an oxygen radical scavenger but also, since it is a cell-permeable sulphydryl compound, readily enters cells and promotes the production of glutathione by furnishing its limiting precursor, L-cysteine [3]. Both mechanisms may provide protection against bleomycin-predicted and leukocyte-mediated cytotoxicity in the lung.

The glutathione status has been studied in patients with idiopathic pulmonary fibrosis by MEYER and coworkers [4, 5] who found a deficiency in total glutathione levels in epithelial lining fluid but not in BALF. Oral treatment with NAC increased the total glutathione levels in the epithelial lining and BALF of patients with idiopathic pulmonary fibrosis, accompanied by an improvement of pulmonary function tests [4, 6]. However, the pathogenic involvement of glutathione deficiency in lung fibrosis induced by bleomycin has scarcely been studied. Intratracheal bleomycin ($5-7.5\ U\cdot kg^{-1}$) did not alter lung non-protein sulphydryl in hamsters but intraperitoneal NAC ($200-400\ mg\cdot kg^{-1}\cdot day^{-1}$ for 13 days) increased lung nonprotein sulphydryl [12]. A tendency was found to lower glutathione levels in BALF of bleomycin exposed rats, as was a significant increase of glutathione in NAC-treated rats. These results suggest that replenishment of lung glutathione may have a role in the beneficial effect produced by NAC against the pulmonary toxicity elicited by bleomycin. However, further studies measuring the reduced and oxidized forms of gluthathione in BALF and lung tissue are required to ascertain the influence of glutathione redox balance in this animal model and in the beneficial effects of NAC.

In addition to replenishment of lung glutathione by NAC, this study also aimed to obtain an indication of the cysteine catabolism in stress conditions as produced by bleomycin administration. Taurine is the last metabolite of cysteine still maintaining the carbon chain [16], and posseses antioxidant properties and other regulatory functions in host defense [25]. Administration of taurine has been found protective *in vivo* in bleomycin-, amiodarone-, and ozone-induced lung injury as well as against oxidant-induced lung epithelial damage *in vitro* [17, 25]. Conversely, taurine levels in BALF are increased after bleomycin or ozone exposure [17, 25], in BALF of asthmatics [26], and in cystic fibrosis sputum [27]. The precise mechanisms of the protective effects of taurine and the explanation for its increased levels in the inflammed lung are still unclear. The beneficial effects of taurine against bleomycin-induced pulmonary damage are ascribed to downregulation of overexpression of the procollagen gene and fibrogenic cytokines, and inhibition of nuclear factor-kappa B activation, effects in which its antioxidant properties appear involved [28].

The present study has confirmed and extended the observation of increased taurine levels in BALF and plasma to bleomycin-exposed rats. NAC further enhanced taurine levels in BALF and plasma of rats receiving bleomycin. Therefore, this amino acid may contribute to the beneficial effect of NAC in this rat bleomycin model. The mechanism underlying this additional augmentation of taurine in the NAC-treated rat is uncertain, but alteration of cysteine catabolism with increased taurine formation under inflammatory conditions has been recently reported [29].

N-acetylcysteine has also been reported to interfere with a number of inflammatory cytokines involved in lung fibrogenesis [14, 30] and to block the *in vivo* activation of nuclear factor-kappa B in rat lungs [23]. Further studies are warranted to elucidate the mechanism of the beneficial effect of *N*-acetylcysteine in

this model, as well as the potential for *N*-acetylcysteine administration as an adjunt therapy for patients with fibrosing alveolitis [6], including that produced during bleomycin treatment.

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