N-acetylcysteine prevents cigarette smoke induced small airways alterations in rats

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ABSTRACT: This study investigated the effect of cigarette smoke exposure and the potential protection N-acetylcysteine (NAC) in rat lungs.

Forty-eight rats were exposed to cigarette smoke (CS) for 10 weeks, without (CS group) or with (CS+NAC group) oral intake of NAC 200 mg·rat⁻¹·day⁻¹, or to fresh air (Control). All rat lungs were assessed in terms of lung function, ventilation distribution (nitrogen, helium and sulphur hexafluoride phase III slopes), and morphometry (airway wall thickening of small, medium and large bronchi).

The small bronchi, defined as the airways with an internal perimeter <1,000 µm showed significantly thicker airway walls in the CS than in the Control group. By contrast, no airway wall thickening was observed in the CS+NAC group with respect to Control. Except for decreased lung volumes and compliance in CS and CS+NAC groups, which were entirely attributable to smaller body weight gain, lung function was indistinguishable from Control. Phase III slopes were significantly increased only in the CS group.

In conclusion, smoke-induced alterations in the rat lungs were reflected in wall thickening of the small bronchi and increased ventilation maldistribution. These smoke-induced morphometric and ventilation distribution alterations were prevented by N-acetylcysteine.

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In humans, a major site of inflammatory action of cigarette smoke has been attributed to the so-called small airways, stimulating a number of studies aimed at characterizing and detecting structural change in the lung periphery [1, 2]. The phase III slope of the N₂ single breath washout (SBW) test became a popular index of small airways alteration since the work by COSIO *et al.* [3] showed its correlation with morphological scores of small airways abnormalities in smokers with normal spirometry. A later SBW study by VAN MUYLEM *et al.* [4], which included helium and sulphur hexafluoride tracer concentrations, also revealed distinct correlations between indices derived from He and SF₆ slopes and airway pathology scores. Due to the fact that the diffusion front is located more peripherally for SF₆ than for He, the respective phase III slopes are indicative of the site where ventilation inhomogeneities occur.

Although animal studies are well-suited for the study of lung function in the diseased lung, the difficulty often resides in reproducing a lesion which is comparable to that encountered in the human lung [5]. Previous studies in rats have led to conflicting results as to whether the cigarette smoke induces emphysematous lesions [6, 7] or not [8, 9] and not all studies provide any information about the

extent to which the nonalveolated airways are affected [10]. Despite these difficulties, rat lungs have been used to investigate the possible protective role of N-acetylcysteine (NAC) over cigarette smoke induced lesions. NAC is an antioxidant and can therefore be expected to change lung oxidant-antioxidant imbalance induced by cigarette smoke which is a source of oxidants. Previous histopathology studies have shown that NAC is indeed able to inhibit [6, 10, 11] and to some extent reverse [12] cigarette smoke induced rat lung alterations. While these studies partly reveal the mechanisms through which NAC prevents damage in different lung zones, its resulting effect in terms of lung function or ventilation distribution is, to the authors' knowledge, nonexistent.

The first aim of this study was to evaluate cigarette smoke induced lesions in rat lungs morphometrically and to assess lung function as well as ventilation maldistribution. For this purpose, those SBW tests (including He and SF₆) that are known to be particularly sensitive to structural alterations, as shown by previous work in normal rats [13] and in rats with induced panacinar and centriacinar emphysema [14, 15] were used. In the present study the authors chose to investigate the formerly described smoke-induced "bronchitis" with absence of emphysema

[10]. The second aim consisted of evaluating whether the cigarette smoke induced alterations can be avoided by simultaneous NAC administration over the smoke-exposed period.

Materials and methods

The male Wistar rats ($n=48$) used in this study were 5 weeks of age at the beginning of the sham/cigarette smoke exposure and NAC (Zambón, Milan, Italy) administration. They were classified into three groups: a Control group, exposed to fresh air ($n=16$); a CS group, exposed to cigarette smoke ($n=16$); and a CS+NAC group, exposed to cigarette smoke and treated with NAC ($n=16$). The rats that were to receive NAC, had NAC $200 \text{ mg rat}^{-1} \cdot \text{day}^{-1}$ mixed with the powdered food beginning 1 day before the first day of smoke exposure until the study day. The administration of NAC through ingestion and the spreading of the daily doses over a morning and an evening food portion was used to ensure constant levels of glutathione in the blood. The daily dose of NAC, $\sim 800 \text{ mg-per kg rat body weight}^{-1}$, was based on doses previously employed by others [6, 10, 11].

Smoke delivery

The smoke delivery system was based on the one previously described by LIU and FUNG [16] and is schematically represented in figure 1. The system includes 16 exposure chambers, a membrane pump and a peristaltic pump (model 7518-00; Cole-Parmer Instrument Company, Vernon Hills, IL, USA) connected to a timer-controlled two-way valve. The membrane pump was used to provide fresh air for dilution (1:10) of the smoke-stream or to supply fresh air during the periods in-between the smoke puffs. Flow rates of the pumps were set to $400 \text{ mL} \cdot \text{min}^{-1}$ (peristaltic pump) and $4,000 \text{ mL} \cdot \text{min}^{-1}$ (membrane pump) to provide sufficient ventilation to the sixteen exposure chambers. The smoke exposure system was set to deliver 12 s smoke puffs alternated by 48 s of fresh air supply, corresponding to a puffing frequency of $1 \text{ puff} \cdot \text{min}^{-1}$.

The 32 rats from the smoke-exposed groups (CS and CS+NAC) were exposed to two cigarettes, three times daily for a total period of 10 weeks. After each exposure to two cigarettes, rats were returned to their cages. The

Fig. 1. – Smoke delivery system (for details see text).

cigarettes used were those from the University of Kentucky (2R1; University of Kentucky, Lexington, KY, USA), the chemical composition of which is described in detail elsewhere [17]. Prior to the actual study, the authors tested the efficiency of the smoke delivery system by measuring in eight rats, blood carboxyhaemoglobin (482 Co-oximeter; Instrumentation Laboratory, Lexington, MA, USA) on two occasions: within 5 min following a two cigarettes smoke-exposure and 48 h after the last two cigarettes smoke-exposure (corresponding to the onset of the actual study).

Rats from the Control group were subject to exactly the same procedure as the rats from the smoker groups (CS and CS+NAC groups), with the only exception that they were exposed to fresh air. Lung function, lung ventilation and morphometrical studies on rats of all three groups were performed 48 h after the last day of cigarette smoke exposure.

Lung function tests

The functional study was performed in a breathing assembly for small animals identical to those previously used for similar studies [13–15]. The rats were anaesthetized with pentobarbital sodium ($50 \text{ mg} \cdot \text{kg}^{-1}$, *i.p.*) and tracheostomized in the cervical region. After that rats were placed into a 1.6-L volume displacement plethysmograph and the tracheal tubing was fitted to a three-way stopcock which allowed communication with the breathing assembly. Rats were paralyzed with pancuronium bromide and then artificially ventilated.

For diffusing capacity measurement, the rat lung was inflated from 0 to $20 \text{ cmH}_2\text{O}$ with a test gas mixture of $0.25\% \text{ C}^{18}\text{O}$ and $10\% \text{ argon (Ar)}$ in air. After 2 s of breath-holding the rat lung was emptied by the mass spectrometer (Marquette Electronics, Milwaukee, WI, USA) at a constant expiratory flow of $1.1 \text{ mL} \cdot \text{s}^{-1}$. The carbon monoxide diffusing capacity (DL_{CO}) and the diffusing coefficient (K_{CO}) were calculated as previously described [15]. The quasi-static pressure-volume curves were obtained by inflating the rat with air to an airway pressure of $30 \text{ cmH}_2\text{O}$, and then having the mass spectrometer deflate the rat lung down to residual volume. Inspiratory capacity (IC) was considered as the lung volume difference between airway pressure 0 and $30 \text{ cmH}_2\text{O}$ on the inflation curve and compliance (C_L) as the steepest slope of the deflation curve. Finally, forced vital capacity (FVC) and forced expiratory flow at 75% of FVC (FEF75) were determined from the flow-volume curves, obtained by inflating the lung with air to $30 \text{ cmH}_2\text{O}$, after which a negative pressure of $-40 \text{ cmH}_2\text{O}$ was applied. The above described lung function tests were performed twice, after which the rats were sacrificed by inflating the lungs to $30 \text{ cmH}_2\text{O}$ with $100\% \text{ N}_2$. After that, functional residual capacity (FRC) was determined using a rebreathing test and ventilation distribution tests were performed within the hour following sacrifice.

Ventilation distribution (single breath washout tests)

SBW tests were performed as previously described [15]. The reference SBW consisted of slowly ($\sim 1 \text{ mL} \cdot \text{s}^{-1}$)

injecting a 4 mL test gas mixture containing 5% He, 5% SF₆ and 90% O₂, starting from FRC, and having the mass spectrometer empty the lungs down to residual volume at a flow rate of 1.1 mL·s⁻¹. Two extra SBW manoeuvres included 4 s or 20 s end-inspiratory breath hold time (t_{BH}). All SBW manoeuvres were performed twice and phase III slopes were determined on all SBW tests. The positive phase III slopes of the lung resident gas N₂ were normalized by mean expired N₂ concentration. The negative phase III slopes of the test gases He and SF₆ were considered in absolute value, and normalized by inspired minus mean expired He or SF₆ concentration.

Morphometry

After removing the cardiopulmonary block, the lungs were fixed by filling them with 10% formalin to an airway pressure of 25 cmH₂O for 24 h [15]. After fixation three lung blocks from three different lobes were taken away for morphometry. From each block 5 µm sections were stained with haematoxylin-eosin. Lung parenchyma was characterized by the mean linear intercept (L_m) as a measure of interalveolar wall distance [15]. The bronchi were visualized by means of a video camera (Hitachi Kp-110®; Hitachi, Tokyo, Japan) adapted to a microscope (Olympus BX50®), using magnifications 4×, 10× or 20× depending on the bronchi size. The images were digitized in 256 tones of grey (fig. 2a) and transformed into a black and white image (fig. 2b), from which bronchial wall area (black area) and lumen area (white area inside the bronchial wall) were determined. Total area was computed as the sum of lumen and wall area [18].

Bronchi which showed a ratio of maximal to minimal internal diameter ≥2 were considered to be cut tangentially and were discarded. All other bronchi were classified according to their internal perimeter [19]: small bronchi (<1,000 µm), medium-sized bronchi (1,000–2,000 µm) and large bronchi (>2,000 µm). This classification method is based on the fact that even when internal and external areas are subject to change, *e.g.* due to airway smooth muscle contraction, the internal perimeter remains constant. Per rat, morphometrical data were obtained from at least 10 large bronchi, 40 medium-sized bronchi and 30 small bronchi.

Statistical analysis

All the data are expressed as mean±SEM. Analyses of variance (ANOVA) and of covariance (ANCOVA) were used for comparison between groups. Multiple range tests (Least Significant Differences method) were used for analysis of differences among means (STATGRAPHICS PLUS; Manugistics Inc., Rockville, MD, USA).

Results

Blood carboxyhaemoglobin measured within 5 min after the cigarette smoke exposure in CS and CS+NAC groups was on average 7.38% of total haemoglobin, higher than that found in the Control rats (2.1%), and similar to that observed in human smokers. By the day of the study,

i.e. 48 h after the last cigarette smoke exposure, carboxyhaemoglobin measured in CS and CS+NAC groups had returned to the level of Control rats.

Functional study

Functional results obtained in Control, CS, and CS+NAC groups are listed in table 1, including also body weights at the beginning and end of the smoke exposure. Although initial body weight was similar between groups, the rats of the cigarette smoke exposed groups (CS and CS+NAC) weighed significantly less than those of the Control groups at the end of exposure period. Both cigarette smoke exposed groups presented significantly lower IC, total lung capacity (TLC: FRC+IC), CL and FVC values than the Control group when using the ANOVA (asterisks in table 1). However, the significant differences for the volumes (IC, TLC and FVC) disappear when applying an ANCOVA with final body weight as a covariable (hash signs in table 1), while the significance for CL persists. Note however, that when CL is normalized to TLC (specific compliance), the ANOVA had not revealed significant differences between the three groups. The slightly lower FRC values in the smoke exposed groups only reached significance in the CS group, independent of whether ANOVA or ANCOVA analyses are used.

Fig. 2. – a) digitized image of a bronchus in 256 tones of grey and 72 pixels per inch of resolution. b) the same bronchus transformed by means of a threshold option into another two-colour image. In black: wall area; in white: lumen area. (Original magnification × 100.)

Table 1. – Mean values ($\pm$ SEM) of body weight and lung function parameters in control, cigarette smoke (CS) and CS+N-acetylcysteine (NAC) groups

	Control group n=11	CS group n=12	CS+NAC group n=12
Initial BW g	119.2 $\pm$ 3.3	133.7 $\pm$ 5.7	123.1 $\pm$ 3.6
Final BW g	374.6 $\pm$ 6.9	325.4 $\pm$ 7.7*	302.5 $\pm$ 5.1* [†]
Weight gain g	255.4 $\pm$ 4.0	198.8 $\pm$ 5.2	177.5 $\pm$ 4.2
Lung Function			
IC mL	17.3 $\pm$ 0.5	15.8 $\pm$ 0.3 [#]	15.6 $\pm$ 0.2 [#]
FRC mL	4.74 $\pm$ 0.16	4.20 $\pm$ 0.10*	4.36 $\pm$ 0.15
TLC mL	22.1 $\pm$ 0.6	20.0 $\pm$ 0.4 [#]	20.0 $\pm$ 0.3 [#]
CL mL $\cdot$ cmH ₂ O ⁻¹	1.29 $\pm$ 0.06	1.07 $\pm$ 0.04*	1.07 $\pm$ 0.04*
CL/TLC cmH ₂ O ⁻¹	0.059 $\pm$ 0.002	0.054 $\pm$ 0.002	0.054 $\pm$ 0.002
FVC mL	18.8 $\pm$ 0.6	17.3 $\pm$ 0.4 [#]	17.2 $\pm$ 0.2 [#]
FEF75 mL $\cdot$ s ⁻¹	39.2 $\pm$ 2.0	35.6 $\pm$ 0.9	35.2 $\pm$ 1.2
FEF75/FVC s ⁻¹	2.09 $\pm$ 0.08	2.06 $\pm$ 0.03	2.05 $\pm$ 0.06
DL _{CO} mL $\cdot$ min ⁻¹ $\cdot$ mmHg ⁻¹	0.218 $\pm$ 0.007	0.203 $\pm$ 0.01	0.204 $\pm$ 0.008
KCO min $\cdot$ mmHg ⁻¹	0.014 $\pm$ 0.001	0.015 $\pm$ 0.001	0.015 $\pm$ 0.001

BW: body weight; IC: inspiratory capacity; FRC: functional residual capacity; TLC: total lung capacity CL: quasistatic lung compliance; FVC: forced vital capacity; FEF75: flow measured at 75% FVC; DL_{CO}: carbon monoxide diffusing capacity; KCO: DL_{CO} per unit alveolar volume. *: significantly different from Control group; [†]: significantly different from CS group discarding Control group, using analysis of variance with $p < 0.05$; [#]: no longer significantly different from Control group when using analysis of covariance with final BW as covariable.

Neither expiratory flow FEF75 nor specific expiratory flow FEF75/FVC were significantly different between any Control, CS, and CS+NAC groups. The same was true for DL_{CO} and KCO.

Ventilation distribution study

The SBW data corresponding to the reference manoeuvre (without breath hold) are represented in figure 3. This figure clearly shows a significant increase of N₂, He and SF₆ slopes in the CS group with respect to Control, while the CS+NAC showed no significant slope difference with respect to Control. The relative slope increase in the CS group was largest for SF₆ (26% with respect to Control, *versus* 19% for He and 17% for N₂). The SF₆-He slope difference was not significantly different between Control, CS and CS+NAC groups. ANCOVA analysis using final body weight and FRC as covariables did not alter the significance of slope differences shown in figure 3. Therefore, final body weight nor FRC (which were significantly lower in CS group; see table 1) had any effect on phase III slope behaviour in the three groups.

Table 2 summarizes the phase III results of all performed SBW manoeuvres (with and without end-inspiration breath hold), showing that the He, N₂ and SF₆ slope increases (which clearly distinguished the CS group from the two other groups for $t_{BH}=0$ s) disappear gradually with increasing breath hold times. For the longest breath hold time ($t_{BH}=20$ s), SF₆-He slope difference changes sign in all three groups, and most markedly so in the CS group. Finally, it can be inferred from table 2 that the relative slope decrease of each gas with breath hold time ran more or less parallel in all three groups, *i.e.*, none of the groups showed a very different breath hold time dependence of He, N₂ and SF₆ slopes.

Morphometry

Analysis of the rat lung parenchyma did not reveal any differences between groups in terms of mean linear inter-

cept: Control: $L_m = 114 \pm 3$ (SEM) μ m; CS: $L_m = 110 \pm 3$ (SEM) μ m; CS+NAC: $L_m = 104 \pm 4$ (SEM) μ m. By contrast, microscopic analysis of the airways revealed differences in terms of airway wall thickness, but only in the small bronchi (*i.e.*, internal perimeter $< 1,000$ μ m). This can be appreciated from figure 4, where small bronchi lumen and wall areas are represented by superimposed bars that add up to the total airway area: for similar lumen area in the three groups, the CS group shows a significantly larger wall area with respect to Control group, whereas airway wall area of the CS+NAC group is indistinguishable from that obtained in the Control group.

Table 2. – Mean $\pm$ SEM normalized nitrogen, helium and sulphur hexafluoride phase III slopes, and the SF₆-He slope difference of the single breath washout tests with 0 s, 4 s, and 20 s end-inspiratory breathhold times (t_{BH}).

	Control group n=11	CS group n=12	CS+NAC group n=12
$t_{BH}=0$ s			
N ₂	0.037 $\pm$ 0.002	0.044 $\pm$ 0.002*	0.039 $\pm$ 0.001 [†]
He	0.046 $\pm$ 0.002	0.054 $\pm$ 0.002*	0.048 $\pm$ 0.002 [†]
SF ₆	0.031 $\pm$ 0.001	0.039 $\pm$ 0.002*	0.034 $\pm$ 0.001 [†]
SF ₆ -He	-0.015 $\pm$ 0.001	-0.015 $\pm$ 0.001	-0.014 $\pm$ 0.001
$t_{BH}=4$ s			
N ₂	0.023 $\pm$ 0.001	0.027 $\pm$ 0.001*	0.024 $\pm$ 0.001
He	0.026 $\pm$ 0.001	0.029 $\pm$ 0.001	0.027 $\pm$ 0.001
SF ₆	0.022 $\pm$ 0.001	0.026 $\pm$ 0.001*	0.022 $\pm$ 0.001 [†]
SF ₆ -He	-0.005 $\pm$ 0.001	-0.004 $\pm$ 0.001	-0.005 $\pm$ 0.001
$t_{BH}=20$ s			
N ₂	0.010 $\pm$ 0.000	0.010 $\pm$ 0.001	0.010 $\pm$ 0.001
He	0.008 $\pm$ 0.000	0.008 $\pm$ 0.001	0.007 $\pm$ 0.001
SF ₆	0.011 $\pm$ 0.000	0.012 $\pm$ 0.001	0.012 $\pm$ 0.001
SF ₆ -He	0.003 $\pm$ 0.000	0.005 $\pm$ 0.000*	0.004 $\pm$ 0.000

*: significantly different from Control group; [†]: significantly different from cigarette smoke (CS) group, $p < 0.05$. Units are expressed in millilitres. NAC: N-acetylcysteine.

Fig. 3. – Nitrogen (N₂), helium (He: □) and sulphur hexafluoride (SF₆; ▨) phase III slopes and SF₆-He (▩) phase III slope differences with IV=4 mL and end-inspiratory breath hold time=0 s for the groups under study. *: significantly different from control group. †: significantly different from cigarette smoke (CS) group ($p<0.05$). CS+NAC: cigarette smoke+N-acetylcysteine.

Discussion

This study suggests that in rats, the cigarette smoke induced alterations of the small bronchi can be avoided by concomitant oral NAC administration. This morphometric observation was also reflected in tests of ventilation distribution, but not in lung function.

Morphometry

The absence of any difference in mean linear intercept between smoke exposed groups (CS and CS+NAC) and Control group, demonstrated that in neither of the rats under study was lung parenchyma affected by 10 weeks of cigarette smoke exposure. KIMMEL and coworkers [8, 20] did not even observe changes in L_m after, respectively, 12 or 14 week cigarette smoke exposure in rats. Inasmuch as cigarette smoke exposure in different animal species provides an indication, hamster lungs did not show significant L_m increases after a 7-week exposure [21], and guinea pig lungs presented a progressive L_m increment following an exposure period of at least 3 months [22]. This indicates that the time of cigarette smoke exposure and the animal employed are crucial for reproducing a human emphysematous lesion, which was clearly nonexistent in the rats under study.

At the level of the large- and medium-sized bronchi, *i.e.* all bronchi with internal perimeter larger than 1,000 μ m, morphometry showed that no airway alterations had occurred in terms of airway wall thickness in either of the smoke exposed groups. Although the bronchi size classification on basis of internal perimeter [19] may have misclassified some severely obstructed medium-sized bronchi as "small bronchi" the cigarette smoke induced lesion clearly occurred essentially at the level of the small bronchi. Small bronchi wall thickness was significantly increased due to cigarette exposure (CS group in fig. 4), an effect which disappeared with NAC treatment in addition to cigarette smoke exposure (CS+NAC group in fig. 4). This observation is very similar to that previously reported in human smokers [3] where the small airways (*i.e.*, internal dia-

Fig. 4. – Lumen area (□; mean $\pm$ SEM) and wall area (▨; mean $\pm$ SEM) of small bronchi (*i.e.*, internal perimeter <1,000 μ m) for the three groups under study. *: wall area of cigarette smoke (CS) group was significantly different from that of control group ($p<0.05$). NAC: N-acetylcysteine. All data are expressed in μ m².

meter <2 mm) were found to be affected, primarily by airway fibrosis and inflammatory infiltrate.

Body weight

Table 1 shows that both cigarette smoke exposed groups had a smaller weight gain than the Control group, the average of CS and CS+NAC groups amounting to 75% of the weight gain of the Control animals. The smaller weight gain of animals exposed to cigarette smoke has been reported by other authors in rats [6, 7] and in guinea pigs [22]. HUBER *et al.* [7] found that rats exposed to cigarette smoke for 6 months only gained 60% of the weight gain of control rats over the same period. BALANSKY *et al.* [6] reported a similar respective weight gain in smoke exposed rats over a 40 days exposure period. BALANSKY *et al.* [6] also reported that NAC did not affect weight gain at all. In the current study, particular attention was given to body weights in the respective groups, because lung function parameters are known to be dependent in part of body weight.

Lung function

Lung function results in table 1 indicated that the smaller lung volumes (IC, TLC, FVC) as well as the decreased compliance (CL) in both cigarette smoke exposed groups could, at least in part, be a consequence of their smaller final body weight. The ANCOVA with final body weight as a covariable showed that this was indeed entirely the case for IC, TLC, and FVC, suggesting that their decreased values were not related to the cigarette smoke exposure itself, but rather due to the relatively smaller weight gain in these groups. The difference in compliance between groups disappeared when divided by TLC, which in itself is affected by body weight.

In summary, the data in table 1 suggest that lung function is hardly affected by 10 weeks of cigarette smoke exposure other than through the indirect effect of body weight. Previous lung function results obtained in cigarette smoke exposed rats without emphysematous lesion are more or less consistent with these findings in

that lung function indices are essentially unaffected by cigarette smoke exposure. For instance, KIMMEL and co-workers [8, 20] and LAI *et al.* [9] did not find any change in lung function of rats following 12–14 weeks cigarette smoke exposure, independent of whether differences in weight gain existed between Control and smoke-exposed groups or not.

Ventilation distribution

Phase III slopes of the SBW were significantly affected by cigarette induced lesions, and this was the case for all three gases in the reference manoeuvre, *i.e.*, $t_{BH}=0$ s (table 2). Since the SBW preinspiratory lung volume (in this study, FRC) is a determinant of phase III slope [23], the authors tested whether the slightly smaller FRC values in the CS group was not at the origin of the observed slope increases. The ANCOVA, using FRC as the covariable, showed that this was not the case. It follows that the observed phase III slope changes can be attributed to cigarette smoke exposure related alterations in ventilation distribution.

In addition to the elevated phase III slopes in the CS group (table 2) the largest relative phase III slope increases were seen for SF₆ (*i.e.*, 26% versus 19% for He). Based on the rat lung anatomical data of RODRIGUEZ *et al.* [24], showing the particularity of acini originating in generations 8–25, simulations predict that the He diffusion front is spread over generations 4–20, while SF₆ front covers more peripheral generations 8–23 [25]. These simulations are based on the diffusion-convection ventilation inhomogeneity theory [26], which accounts entirely for the experimental SBW slopes in rat lungs [13, 27]. This theory also predicts that if structural alteration is to occur at the level of the diffusion front of a given test gas, this test gas phase III slope will be most affected by it.

The small bronchi, with internal perimeter <1,000 μm , correspond to the airways from the 7th generation onwards [24] which corresponds to the onset of the SF₆ diffusion front. Had the morphometrical changes occurred in the large-sized bronchi, proximal to the SF₆ diffusion front, a marked He slope increase with hardly any change in SF₆ slope would be expected. Conversely, the spread of the He and SF₆ diffusion fronts between generations 4–20, and 8–23, respectively, implies that both slopes will be affected to some extent by changes occurring beyond generation 7, but slightly more so for SF₆. In this respect the respective He and SF₆ slope responses to cigarette smoke exposure are consistent with the morphometric observation of small bronchi alteration.

Finally, the SBW tests including end-inspiratory t_{BH} (table 2) showed that the percentage decrease of N₂, He and SF₆ slopes as a function of t_{BH} with respect to the manoeuvre without post-inspiratory breath-hold ($t_{BH}=0$ s) was similar in all groups. This indicates that the very structure of the rat lung did not change in essence, contrasting with what the authors have previously obtained in emphysematous rat lungs [15]. The morphometric changes observed in this study are relatively mild alterations of the small nonalveolated airways in a supporting lung structure which basically remains unaltered. This is also consistent with the fact that SF₆-He never reverses sign

between Control and CS rats (for any given t_{BH}) as it did in emphysematous with respect to normal rat lungs [15].

The effect of N-acetylcysteine

The group receiving NAC in addition to cigarette smoke did not present a worsening of ventilation distribution in terms of alveolar slopes and its behaviour as a function of t_{BH} was similar to that obtained in the Control group. So, not only does the protective effect of NAC show through the morphometric data, but also through resulting ventilation distribution data. A major question arising from these observations concerns the mechanism by which NAC exerts its protective effect over the airways affected by cigarette smoke. On the basis of the current data, the authors speculate that since the lung parenchyma remained unaffected, it is unlikely that the proposed oxidative effect of free radicals over lung parenchyma [28] would explain the observed lung protection. The significant airway wall thickening in the CS group and normal airway wall thickness in the CS+NAC group, favours the hypothesis of the anti-inflammatory role that has been attributed to NAC [29]. It has been demonstrated that NAC suppresses nuclear factor- κB activation [29], a factor that stimulates the transcription of many cytokines participating in the inflammatory-repair reaction. So, NAC could act at this level, modulating the production of these mediators and definitively regulating inflammation and tissue repair.

In conclusion, exposure of rats to cigarette smoke induced clear-cut morphological alterations of the small bronchi and a worsening of ventilation distribution. Conventional lung function was not able to detect the small bronchi wall thickening. Morphometry and ventilation distribution remained normal when cigarette smoke exposed rats had received N-acetylcysteine.

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