Effect of cigarette smoke on bronchial reactivity

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OBJECTIVE: To compare the effects of a brief low level exposure to cigarette smoke in rats with known low (Sprague-Dawley) and high (Fisher) airway responsiveness, to test the hypothesis that airways reactivity influences the severity or duration of pulmonary function alterations after cigarette smoke exposure.

METHODS: Baseline pulmonary function tests and methacholine dose response tests were conducted in 10 Sprague-Dawley and 10 Fisher rats. On the following day, the animals were reanaesthetized, and breathed for 1 min from a 2 L chamber into which 25 mL of fresh cigarette smoke had been injected, followed by a second set of pulmonary function and methacholine response tests; a final set was performed two weeks later.

RESULTS: Sprague-Dawley rats were larger, with larger lung volumes, compliance and flow rates, but Fisher rats showed a fourfold higher airway reactivity. Although nei-

ther resistance nor response to methacholine changed in either strain of animal, Sprague-Dawley rats showed an increase in residual volume post smoke, which was not sustained over two weeks, and sustained small increases in vital capacity, total lung capacity and static lung compliance, with a sustained decrease in forced expiratory volume in 0.1 s, while Fisher rats showed only a small sustained increase in functional residual capacity.

CONCLUSIONS: Although there are marked differences in pulmonary function between the two different strains of rats, increased airways responsiveness per se does not make the animal more sensitive to the acute effects of cigarette smoke, and the effects of cigarette smoke on pulmonary function are not necessarily related to increased airway resistance. Pulmonary function alterations seen after brief cigarette smoke exposure may be sustained for a relatively long period of time. (*Pour le résumé, voir page 232*)

Key Words: Airways responsiveness. Cigarette smoke. Pulmonary function

Effet de la fumée de cigarette sur la réactivité bronchique

OBJECTIF : Comparer les effets d'un faible et bref niveau d'exposition à la fumée de cigarette chez des rats Sprague-Dawley connus pour avoir une faible réactivité bronchique et des rats Fisher ayant une forte réactivité bronchique ; vérifier si la réactivité bronchique influence la gravité ou la durée des atteintes de la fonction pulmonaire après une exposition à la fumée de cigarette.

MÉTHODES : Des épreuves de fonction respiratoire de base et des tests de provocation bronchique à la méthacholine ont été menés chez 10 rats Sprague-Dawley et 10 rats Fisher. Le jour suivant, les animaux ont été réanesthésiés, et ont respiré pendant une minute de l'air provenant d'une chambre de 2 L dans laquelle 25 mL de fumée de cigarette avaient été fraîchement injectés. Ensuite, on a procédé à une deuxième épreuve de fonction respiratoire et à un second test de provocation bronchique à la méthacholine. Une série finale de tests a été pratiquée deux semaines plus tard.

RÉSULTATS : Les rats Sprague-Dawley étaient plus gros, avec des volumes pulmonaires, une compliance et des débits plus grands.

Cependant, les rats Fisher ont démontré une réactivité bronchique 4 fois supérieure à celle des rats Sprague-Dawley. La résistance ou la réponse à la méthacholine sont restées les mêmes chez les deux races de rats ; cependant, les rats Sprague-Dawley présentaient une augmentation du volume résiduel suite à leur exposition à la fumée qui ne subsistait plus après deux semaines. Ils présentaient également de faibles augmentations soutenues de la capacité vitale, de la capacité pulmonaire totale et de la compliance pulmonaire statique ainsi qu'une diminution soutenue du volume expiratoire maximum/seconde. Les rats Fisher présentaient uniquement une petite augmentation soutenue de la capacité résiduelle fonctionnelle.

CONCLUSIONS : Bien qu'il y ait des différences appréciables de la fonction respiratoire entre les deux races de rats, l'augmentation de la réactivité bronchique *per se* ne signifie pas que l'animal est plus sensible aux effets aigus de la fumée de cigarette, et les effets de la fumée de cigarette sur la fonction respiratoire ne sont pas nécessairement liés à l'augmentation de la résistance des voies aériennes. Les altérations de la fonction respiratoire observées après une brève exposition à la fumée de cigarette peuvent durer pendant une période relativement longue.

BRONCHIAL HYPERREACTIVITY MAY BE AN IMPORTANT determinant of disease susceptibility in chronic obstructive pulmonary disease (COPD) (1,2). Longitudinal studies have indicated that airways hyperreactivity (3) and lack of response to bronchodilators (3,4) are important predictors of an increased rate of decline in forced expiratory volume in 1 s (FEV₁), independent of the effects of smoking. Similarly, a recent study of young cigarette smokers (5) has shown that the presence of wheezing, probably indicative of lung hyperresponsiveness, is predictive of a progressive loss of ventilatory function. A recent multicentre clinical trial (Lung Health Study) showed that current smokers with functional evidence of early COPD had nonspecific airways hyperresponsiveness that was related to the baseline values for lung function (6). Tashkin and colleagues (7) have recently shown a dose-dependent effect of tobacco smoking with airways responsiveness, which appears to be independent of the effects of lung function. This latter study would support the concept that cigarette smoke has a primary effect on airways hyperresponsiveness.

Passive smoke exposure has also been related to increased airways responsiveness, both in children at home (8) and in adults at their workplace (9). Mechanisms of induction of airway reactivity in these studies are unclear, but it has been suggested that the process may be related to an inflammatory response in the airways. Although, in many animals, an acute exposure to cigarette smoke may be associated with an immediate increase in airways resistance (10-13) and appears to make the airways more sensitive to standard doses of acetylcholine (14) or methacholine (13,15), the mechanism is disputed.

In the present study, we wished to ascertain whether innate airways hyperreactivity would cause animals to have a greater sensitivity to a brief low level exposure to cigarette smoke, and thus result in sustained increases in airways resistance or methacholine responsiveness. To test this hypothesis,

two groups of rats with different degrees of airways responsiveness were selected. We chose the Fisher rat for one group since this strain is known for its sensitivity to standard bronchoconstrictive agents (16), and it is also known that these animals have significantly more airway wall smooth muscle than is found in a less responsive strain of rat (16). Sprague-Dawley rats were chosen as a normoresponsive control.

ANIMALS AND METHODS

Groups of 10 Sprague-Dawley and 10 Fisher rats were used. On day 1, baseline pulmonary function tests and methacholine challenge were performed as documented below; on day 2, the animals were re-anesthetized and were allowed to breathe for 1 min from a 2 L chamber containing 25 mL fresh cigarette smoke. This dose was chosen from an initial study using Sprague-Dawley rats that demonstrated that this smoke concentration would produce an initial rise in airways resistance that would not be sustained after 10 mins. Ten minutes after the smoke inhalation, repeat pulmonary function tests and methacholine challenge were done. A final set of pulmonary function tests and methacholine challenge were performed two weeks after initial baseline values. To determine that methacholine responsiveness did not change due to the experimental procedure, a control group of five Sprague-Dawley rats was studied using the same protocol.

Pulmonary function tests were performed as follows: the rats were anesthetized with a combination of intraperitoneal diazepam (5 mg/kg) and intramuscular fentanyl droperidol (Janssen Pharmaceutical) (0.2 mg/kg). After intubation with a 14-gauge intravenous cannula using a pediatric laryngoscope and a tilted table, the rats were placed in a pressure sensitive 7.5 L small animal plethysmograph and ventilated at 80 breaths/min at a tidal volume of approximately 2 mL. A water filled esophageal tube (size PE 240, Intramedic catheter, Clay Adams, New Jersey) with a multiholed tip was used to measure pleural pressure; transpulmonary pressure was

TABLE 1
Baseline values and pulmonary function results for Sprague-Dawley and Fisher rats

Pulmonary function	Sprague-Dawley rats			Fisher rats		
	BL	S	PS	BL	S	PS
RV (mL)	4.1±1.7	5.8±1.4*	5.5±1.9	4.3±1.5	4.7±1.4	4.3±1.5
FRC (mL)	9.0±0.5	9.3±0.7	9.0±0.5	7.3±0.2 [†]	8.2±0.2*	7.5±0.2*
VC (mL)	19.4±1.3	20.2±1.5*	20.9±1.8*	12.5±1.1 [†]	13±0.9	12.8±1.6
TLC (mL)	23.4±2.1	26±1.8*	26.4±1.3*	16.8±1.3 [†]	17.6±1.1	17.1±1.8
Cst (mL/cm H ₂ O)	0.78±0.27	1.01±0.3*	1.04±0.29*	0.54±0.18 [†]	0.44±0.21	0.54±0.28
RES (cm H ₂ O/mL)	0.12±0.18	0.11±0.07	0.09±0.03	0.16±0.06	0.11±0.06	0.11±0.04
R200 (mg/mL)	1.77±1.41	1.5±1.15	1.20±0.35	0.43±0.35 [†]	0.17±0.08	0.38±0.30
FEV _{0.1} (mL)	5.2±1.1	4.1±1.1*	3.3±1.0*	4.5±0.8	4.1±0.9	2.7±1.1
FEF ₂₅₋₇₅ (mL/s)	86±37	108±21	107±9	77±13	79±18	82±12
PEF (mL/s)	140±15	144±17	149±15	106±10 [†]	107±16	105±12

*Significantly different from baseline values. [†]Significantly different from Sprague-Dawley rats' baseline values. BL Baseline; Cst Static lung compliance; FEF Forced expiratory flow between 25 and 75% forced vital capacity; FEV_{0.1} Forced expiratory volume in 0.1 s; FRC Functional residual capacity; PEF Peak expiratory flow; PS Two weeks post smoke; R200 Methacholine dose that doubles the baseline resistance; RES Dynamic airways resistance; RV Residual volume; S 10 mins after smoke exposure; TLC Total lung capacity; VC Vital capacity

calculated as the difference between mouth and pleural pressure. Results were analyzed using the RAYTEC computer-generated pulmonary function analysis system (Fine Science Tools). Functional residual capacity (FRC) was measured by airway occlusion at end-expiration, following which the rats were given supplementary doses of fentanyl droperidol and were rendered apneic by hyperventilation. Pressure volume curves were constructed from values obtained by deflation to -30 cm H₂O, inflation to +30 cm H₂O and deflation to -30 cm H₂O. Two such inflation-deflation manoeuvres were performed before measurement of the curve (with concurrent calculation of lung volumes expiratory reserve volume, residual volume, vital capacity and total lung capacity [TLC]). Static lung compliance was calculated between FRC and FRC plus 10 cm transpulmonary pressure. A flow volume curve was constructed from values at inflation to +30 cm H₂O and at rapid deflation at -50 cm H₂O pressure. From this curve, forced vital capacity, forced expiratory flow, forced expiratory flow between 25 and 75% forced vital capacity (FEF₂₅₋₇₅), FEV_{0.1} and peak expiratory flow were calculated. The flow volume curve was plotted by determining the flow at each of the volumes between 30 and 95% TLC.

The methacholine challenge was performed on spontaneous breathing rats, using a methodology adapted from Martin and colleagues (17,18). An Acorn nebulizer (Trudell Medical) was used with 7 L/min air flow, which delivered the particles into a 1 L vented breathing chamber attached to the endotracheal tube. The rat was allowed to breathe spontaneously from this chamber for 2 mins, and after a 30 s period of room air breathing, resistance was measured and averaged over approximately 10 breaths. Baseline resistance was measured after delivery of normal saline; then doubling methacholine concentrations from 0.05 to 0.8 mg/mL were used, followed by concentrations incrementing by 0.25 mg/mL from 1 to 5 mg/mL. R200 was calculated from the dose response curve as the concentration of methacholine required to double baseline resistance.

Following the pulmonary function tests, the animals were

kept warm, allowed to recover from the anaesthesia and were extubated. They were housed in a laminar hood in standard rat cages with paper pellets as bedding, and were allowed free access to rat chow and water.

The data were first normalized by log transformation. All analyses were done with the SYSTAT system (SYSTAT Inc, Illinois). Unpaired *t* tests were used to test for strain differences at each point in time. Paired *t* tests were performed to ascertain whether a change from baseline had occurred within an animal group. Two-way repeated measures analysis of variance was used to compare level of pulmonary function both within and between the two animal strains.

RESULTS

The control animals had mean ± SD R200 and airway resistance, respectively, of 0.85±0.30 and 0.06±0.02 at baseline, 0.73±0.30 and 0.06±0.02 post sham smoke, and 1.16±0.35 and 0.05±0.01 two weeks post sham smoke. Paired *t* tests showed no difference between time points.

There was a marked difference between the baseline values of the Sprague-Dawley and Fisher rats (Table 1). Although the rats had similar baseline resistance, the Fisher animals demonstrated an approximately fourfold greater responsiveness to methacholine than the Sprague-Dawley rats (*P*<0.003). As shown in Table 1 and Figure 1, Fisher rats had smaller FRC (*P*<0.001), vital capacity (*P*<0.001) and TLC (*P*<0.001), with a downward shift of the pressure volume curve (*P*<0.001) and decreased static lung compliance (*P*<0.01). Baseline flow volume curves (Figure 2) had significantly reduced flows at comparable percentage TLC compared with those found in the Sprague-Dawley animals (*P*<0.002), and peak flows were reduced (*P*<0.001).

In the Sprague-Dawley rats, smoke produced an increase in residual volume (*P*<0.001), which did not persist after two weeks. This was associated with an increase in static lung compliance (*P*<0.03), and increases in vital capacity (*P*<0.04) and TLC (*P*<0.001), with an upward shift in the pressure volume curve (*P*<0.006), all of which persisted over two

Figure 1) Baseline pressure volume curve for Sprague-Dawley (solid line) and Fisher (hatched line) rat strains. Values are mean $\pm$ SD for each group as a whole. There is a significant ($P < 0.001$) difference between the curves. TLC Total lung capacity

Figure 2) Baseline flow volume curve for Sprague-Dawley (solid line) and Fisher (hatched line) rat strains. Values are mean $\pm$ SD for each group as a whole. There is a significant ($P < 0.002$) difference between the curves. TLC Total lung capacity

weeks ($P < 0.04$, $P < 0.002$, $P < 0.005$ and $P < 0.001$, respectively). Although the expiratory flow volume curve was not altered after smoke, the FEV_{0.1} was significantly diminished ($P < 0.02$), and there was a further decrease after two weeks ($P < 0.01$).

In the Fisher rats, the FRC was significantly increased post smoke ($P < 0.001$), and this change was persistent over the following two weeks ($P < 0.02$). No other alterations in the lung volumes or pressure volume curve were found; similarly, the flow volume curve and flow rates remained stable.

DISCUSSION

We devised the present experiments to answer the following questions. First, are there any differences between the acute reactions of a normoreactive (Sprague-Dawley) and a hyperreactive (Fisher) strain of rat to a minimal dose of cigarette smoke? Second, is there any long term effect on the pulmonary function or methacholine reactivity of exposure to a brief, low intensity, dose of cigarette smoke? We found that, regardless of initial airways responsiveness, this minimal exposure did not increase sensitivity to methacholine. It did, however, alter pulmonary function, and several of these alterations were persistent. Surprisingly, the pulmonary function changes were primarily seen in the less responsive Sprague-Dawley animals, with only the FRC being altered in the Fisher rats.

In humans, short term inhalation of sidestream smoke will produce a dose-related increase in symptoms and a significant decrease in pulmonary function in patients with increased methacholine sensitivity (19). Other studies in animals have shown that cigarette smoke will increase airways resistance (11-14). Xu and colleagues (15) exposed rats to three cigarettes per day, five days per week, for 15 weeks. They found that this regimen did not alter baseline resistance, but decreased the R200 and allowed the detection of a resistance plateau in the methacholine dose response curve. Although they were able to demonstrate increases in TLC, they could not relate the changes in responsiveness to alterations

of lung elasticity. Our study suggests that changes in lung elasticity, lung volumes and FEV_{0.1} may occur very early in the genesis of cigarette smoke-induced lung disease, pre-dating alterations in airway responsiveness. The nature of the alteration of lung elasticity is unclear; although we have previously found that exposure to cigarette smoke will produce initial destruction of lung collagen matrix (20), the smoke exposure in that study was of much greater intensity and of longer duration than that of the present study.

We do not consider it likely that the changes in pulmonary function shown in the present study can be attributed to the effects of inflammation. Induction of inflammation by the smoke would preferentially affect the airways and be reflected in increased resistance or increased bronchial responsiveness (10,14), changes not found in our study. In support of this conclusion are the studies of Xu et al (15) and Nishikawa and colleagues (13). Neither of these groups could demonstrate evidence for inflammation after a much more intensive exposure to cigarette smoke.

In humans, airway structure has been suggested to be an important predisposing factor for airways hyperresponsiveness, and may explain the female sex bias for increased airways reactivity in smokers with mild COPD (21). In the present study, we found marked differences in the pulmonary function of Sprague-Dawley and Fisher rats, all lung volumes and flow rates being higher in the former. Some of the differences may be attributable to differences in size as reflected in their weight; Sprague-Dawley animals weighed 475 ± 39 g at baseline while the Fisher animals weighed 297 ± 17 g. The Fisher rats grew only 36 ± 6 g compared with 72 ± 6 g seen in the Sprague-Dawley animals, suggesting different underlying growth rates. Possible differences in the lung structure in the two strains could also explain the differences in pulmonary function. Eidelman et al (16) showed that the Fisher rats have different airway structure with increased amounts of airway smooth muscle compared with the less responsive Lewis rats, and suggested that they may explain

the differences in innate airway responsiveness. It is also possible that these differences in lung structure could additionally explain the paucity of cigarette smoke-induced changes in pulmonary function in the Fisher compared with the Sprague-Dawley rats.

CONCLUSIONS

Although a brief, low intensity dose of cigarette smoke does not alter airways resistance or responsiveness, it does produce alterations in pulmonary function, some of which are

persistent, at least in the short term. The changes are apparently not related to airways responsiveness and are more pronounced in the normoresponsive rats. The increased airways responsiveness that can be produced by exposure to cigarette smoke appears to be separate from other alterations in pulmonary function.

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