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Chronic Sidestream Cigarette Smoke Exposure Causes Lung Injury in Rabbits

Key Words
Passive smoke exposure
Lung permeability
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Abstract
The effects of sidestream cigarette smoke (SSCS) (a 15-min exposure per day for 20 days) were determined on markers of lung injury in New Zealand white rabbits (n = 9) and a control group (n = 6). The SSCS consisted of air and smoke which were aspirated by syringe from a funnel inverted over a lit cigarette. The rabbits were placed in an environmental chamber into which 3 liters of SSCS were injected over a 15-min period each day. Chronic SSCS caused an increase ($p < 0.05$) in pulmonary epithelial clearance (k) of technetium-labeled diethylenetriamine pentaacetate (^{99m}TcDTPA); k = 0.83 (± 0.07) for the SSCS-exposed group and 0.66 (± 0.02) for the control group. This increase in lung permeability was accompanied by an increase in bronchoalveolar lavage (BAL) white cell count; SSCS = 83,353 (± 11,954) cells/mm^3 BAL fluid versus control = 16,450 (± 6,683) cells/mm^3 BAL fluid and an increase in BAL leukotriene E_4; SSCS = 742 (± 285) pg/ml BAL fluid compared with 76 (± 2) pg/ml BAL fluid for controls. Cultured SSCS alveolar macrophages (AMs) produced more superoxide (O_2^-); 2.4 (± 0.8) nmol $O_2^-/10^6$ AMs versus 0.4 (± 0.2) nmol $O_2^-/10^6$ AMs for controls after incubation for 18 h with 10 μg/ml lipopolysaccharide. Electron microscopy demonstrated that the airway mucosa of SSCS rabbits was infiltrated by eosinophils, and light microscopy showed focal clusters of neutrophils in perivascular and capillary spaces. It is concluded that SSCS exposure can induce lung injury.

Introduction

Environmental tobacco smoke (ETS) is a complex mixture of smokes that include sidestream cigarette smoke (SSCS), smoke coming through the cigarette, both filtered and unfiltered mainstream smoke (MS) and cigarette smoke exhaled from smokers' lungs. ETS has the potential to be a major health problem. Urban residents spend more than 90% of their time indoors and consequently may be exposed to ETS [1]. Epidemiologic evidence suggests that ETS exposure may increase the risk of lung cancer [2–4].

A human study demonstrates that ETS causes injury to the lungs: in children who lived in a town with a lead

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smeltry, a significant correlation between higher blood lead levels and increased ETS exposure was found [5]. This finding demonstrates that ETS exposure may increase pulmonary permeability and ultimately allow environmental toxins into the systemic circulation. There is no epidemiologic evidence of the effect of chronic SSCS on human lung function.

Of the several components of ETS, only MS has been examined in any great detail. Chronic exposure for 28 days to 10 puffs of cigarette smoke drawn through the cigarette caused alterations in the prostacyclin (PGI_2): thromboxane (TxB_2) ratio in rat aortic tissue [6]. Acute exposure to large amounts of MS cigarette smoke caused an increase in both plasma and bronchoalveolar lavage (BAL) concentrations of 6-keto-$PGF_{1\alpha}$ (stable metabolite of PGI_2) as well as an increase in pulmonay epithelial clearance of technetium-labeled diethylenetriamine pentaacetate (^{99m}TcDTPA; physical half-life 6.02 h, MW = 492 daltons) [7].

Very little information is available regarding the effects of chronic SSCS on lung function. Thus, the present paper reports the effects of 20 days of SSCS exposure on pulmonary epithelial permeability and lung eicosanoid metabolism in rabbits.

Methods

Animal Selection and Exposure Regimen

Adult New Zealand white rabbits of either gender with a weight range of 2.5–3.6 kg were randomly assigned to a control group (n = 6) or an SSCS-exposed group (n = 9).

Rabbits in the SSCS group were exposed for 20 consecutive days to 3 liters of SSCS/day for 15 min/day. SSCS was generated by the combustion of 2 unfiltered cigarettes of a commercially available brand. SSCS was generated in the following manner: the first cigarette was lit and 1 puff of smoke was drawn from the cigarette by the investigator. The lit cigarette was placed upright in a clamp 2.5 cm below the bottom edge of an inverted 220-cm^3 funnel. The total mass concentration of SSCS particulate matter delivered to the rabbits was 2 mg/exposure, as measured by a cascade impactor (Anderson Samplers, Atlanta, Ga., USA). The SSCS-air mixture was taken from the top of the funnel with a 60-ml plastic syringe in a 3-second draw procedure in 50-ml aliquots and immediately injected into a 1 cm diameter port located on the top surface of a 24 × 36 × 39 cm^3, 33.7 liter total volume, plexiglass environmental chamber. The plexiglass environmental chamber was a sealed system except for the injection port and a 4.5 cm diameter port located on one side of the chamber. No device was used to mix the air inside the chamber. The SSCS particles inside the chamber were found to have a mass mean aerodynamic diameter of 0.6 μm with a standard geometric deviation of 2.3, as measured with a cascade impactor.

The SSCS exposure regimen was standardized in the following manner. The SSCS rabbit was placed into the environmental chamber for the 15-min exposure period. Fifteen 50-ml aliquots of SSCS-air were injected into the chamber, loose ashes were dislodged from the cigarette tip, and another 15 50-ml aliquots were injected into the chamber. This process was then repeated with a fresh cigarette. The delivery of 3 liters SSCS-air into the environmental chamber was consistently accomplished in 12–13 min. However, each SSCS rabbit was kept in the chamber a total of 15 min, then removed from the chamber, and returned to the animal facility. The chamber was then washed to prevent the accumulation of nicotine and other cigarette-derived material. Control rabbits were time-matched with a corresponding SSCS rabbit over the 20-day exposure period, but were kept in their cages in the animal facility until the day of the experiment because it was uncertain whether washing the environmental chamber would remove all cigarette-derived material. The last SSCS exposure was at least 18 h before the experimental procedure.

Arterial blod samples were withdrawn immediately after a standard SSCS exposure in 4 rabbits to determine carboxyhemoglobin percentage (COHb%). COHb% was measured with a co-oximeter (Model 482; Instrumentation Laboratories, Lexington, Mass., USA). Because this instrument is not designed to measure COHb in rabbits, a standard CO curve was constructed by the method of Siek and Rieders [8]. The data points had a linear regression coefficient (r = 0.99) with the origin forced through zero.

Animal Preparation

Each rabbit was anesthetized intramuscularly with 50 mg/kg ketamine HCL (Parke, Davis & Co., Detroit, Mich., USA), 8 mg/kg xylazine (Miles Laboratories, Elkhart, Ind., USA) and 1 mg/kg acepromazine maleate (Aveco, Fort Dodge, Iowa, USA). A tracheostomy was performed with an endotracheal tube (ET; inner diameter 3 mm, length 14.5 cm) placed 2 cm above the carina. Rabbits were paralyzed by an intravenous injection of 20 mg gallamine triethiodide (Flaxedil; Lederle Laboratories, Pearl River, N.Y., USA) and mechanically ventilated (Harvard Apparatus, South Natick, Mass.) with a tidal volume (V_T) of 14 ml/kg and a frequency of 40 breaths/min to maintain a $PaCO_2$ of 35–45 mm Hg. Gamma radiation was measured with two Ludlum portable scaler rate meters (Model 2200; Ludlum Co., Sweetwater, Texas, USA) with scintillation probes (Model 44-2). These rate meters have a linear range of 0–500,000 counts/min. One probe was placed over the right lung in the sagittal plane and another was positioned over the medial aspect of the right thigh, excluding the bladder. Great care was taken to position the lung probe at the site of the greatest right-lung sounds [7, 9, 10].

Procedures

^{99m}TcDTPA was aerosolized into each rabbit's lungs for 3 min. One millicurie ^{99m}TcDTPA was mixed in 2 ml sterile normal saline and aerosolized with a DeVilbiss Pulmo Sonic nebulizer (Model 25, Somerset, Pa., USA). The nebulizer was inserted between the distal end of the inspiratory limb of the nebulizer circuit and the ET using a separate set of ventilator tubing. This nebulizer circuit produced droplets with a mean mass aerodynamic diameter of 1.58 (± 0.7) μm, as measured with a cascade impactor. The output of the nebulizer circuit was 0.5 ml/min at a tidal volume of 30 ml and a rate of 40 breaths/min.

The Division of Nuclear Medicine prepared ^{99m}TcDTPA, using standard kits (Medi-Physics, Emeryville, Calif., USA). Binding of ^{99m}Tc and DTPA was consistently greater than 97%, as determined by thin-layer paper chromatography. It had been shown previously that the aerosolization, pulmonary uptake and passage of

^{99m}TcDTPA into the rabbit's urine does not affect binding of ^{99m}Tc and DTPA [10].

Pulmonary clearance of ^{99m}TcDTPA was established over a 15-min counting period, beginning immediately after aerosolization. Gamma counts were recorded for the first 30 s of every minute. The ^{99m}TcDTPA counts were used to determine percent clearance/minute (k), and these k values were corrected for background radiation. At the end of the study session, the rabbits were killed by incision of the abdominal aorta. The cardiac lobe of the lung was tied off and removed for pathologic studies, and the remainder of the lungs were immediately removed for BAL.

The BAL was performed by cutting off the connector of the ET and injecting saline through its tube portion. Washes were repeated twice with 30-ml aliquots of sterile 0.85% saline solution [11]. For subsequent analysis, the washes were pooled. Recovery rate was consistently greater than 75%. A 35-ml portion of the BAL fluid was used for collection of alveolar macrophages (AMs). The remaining 10 ml of BAL fluid was decanted into a chilled polypropylene tube and spun at 275 g for 10 min at 4 °C. The supernatant was decanted from the cell pellet, loaded and washed on octadecylsilyl Sep-Pak cartridges (Waters Corp., Milford, Mass.), and stored at – 70 °C without preservative until used for assay.

BAL concentrations of leukotrienes B_4, C_4 and E_4, and 6-keto-$PGF_{1\alpha}$, PGE_2 and TxB_2 (stable metabolite of TxA_2) were determined. Leukotriene and prostaglandin concentrations were measured by radioimmunoassay techniques using standard kits (Du Pont NEN Research Products, Inc., Boston, Mass.). All samples were run in duplicate and averaged to obtain a mean value.

Results of eicosanoid radioimmunoassays were confirmed by high-pressure liquid chromatography (HPLC). The HPLC isocratic solvent mixture was 30% methanol, 69.98% HPLC-grade water, and 0.02% phosphoric acid titrated to a pH of 5.7 with ammonium hydroxide. A Waters Nova-Pak C^{18} column was used in the analyses with a flow rate of 1.5 ml/min: 15-µl aliquots of LTB_4, LTC_4 and LTE_4 standards from Du Pont radioimmunoassay kits were used to determine the HPLC profile of these leukotrienes. The retention time of leukotrienes and percentage total area of HPLC peaks at that time were as follows: LTB_4, 0.83 min and 74.5%; LTC_4, 1.01 min and 75.6%, and LTE_4, 0.66 min and 98.2%. Thirty-microliter aliquots of BAL fluid from 3 SSCS rabbits were subjected to the same procedures, and the major peak occurred at an average of 0.69 (± 0.003) min and 94.6 (± 4.6)% total area of HPLC peaks at this time. In addition, a portion of BAL fluid obtained from 2 SSCS rabbits was immediately frozen to confirm that the radioimmunoassay results were not skewed by the degradation of LTC_4 to LTE_4 during the 10-min centrifugation period.

The BAL cell pellet was resuspended in sterile normal saline, and total cell counts were determined using a hemocytometer. Approximately 2×10^5 cells were fixed onto each of several glass slides in a Shandon Cytospin II centrifuge (Astmoor, England, UK). Differential cell counts were performed using a Wright-Giemsa-stained slide under light microscopy.

Pathologic Procedures

Representative sections of the cardiac lobe were processed with formalin. In addition, portions of the cardiac lobe were taken for light and electron microscopy. The tissue for electron microscopy was fixed in 4% glutaraldehyde for 2 h, transferred to cacodylate buffer, postfixed in osmium tetroxide, imbedded in Epon, and stained with uranyl acetate-lead citrate. Sections were examined with a JEOL 100CX electron microscope (× 19,000 maximal magnification). There was no evidence of respiratory infection in lung sections of the rabbits used for pathologic studies.

AM Superoxide Production Studies

AMs were obtained as described previously. AMs (1×10^6) from control and SSCS rabbits were plated into 24-well tissue culture plates (Falcon; Becton-Dickinson, Mountain View, Calif.) in RPMI containing 5% fetal calf serum, allowed to adhere for 2 h, and nonadherent cells were removed. Wells were filled with medium alone, or with fresh medium containing 10 µl of lipopolysaccharide (LPS; Sigma Chemical Co., St. Louis, Mo., USA) for 18 h to prime the AMs. Medium was removed, the cells washed 3 times with PBS, and 1.5 ml of reaction mixture containing 80 µmol of horse heart ferricytochrome c type III (Sigma), with or without 1 µg/ml of phorbol myristate acetate (PMA; Sigma), was added to each well. Control wells contained reaction mixture, with the addition of superoxide dismutase (SOD; Sigma). After incubation at 37 °C for 1 h, reaction mixture was removed, centrifuged at 10,000 *g* for 5 min at 4 °C, and placed on ice. OD^{550} was measured, and nanomoles of superoxide (O_2^-) calculated using the formula (OD^{550} sample – OD^{550} blank) / 21.1.

Statistical Analysis

Mean (± SEM) data were calculated for the control and SSCS rabbit groups. The Mann-Whitney test was used to determine significant ($p < 0.05$) differences between the groups [12].

Results

Chronic SSCS exposure caused an increase in pulmonary epithelial permeability that was present at least for 18 h after the last exposure to smoke. There was a significant increase in SSCS ^{99m}TcDTPA k value of 0.83 (± 0.07) versus a control k of 0.66 (± 0.02). In addition, SSCS rabbits demonstrated an increase in BAL LTE_4 levels (table 1) that were confirmed by HPLC. In addition, there were no differences between the eicosanoid concentrations in the immediately frozen BAL fluid and the same BAL fluid after the 10-min centrifugation period, indicating that there was no degradation of eicosanoids during centrifugation.

The SSCS rabbits had an increase in BAL total white cell count, 83,353 (± 11,954) cells/mm^3 BAL fluid compared to controls 16,450 (± 6,683) cells/mm^3 BAL fluid, but there were no changes in BAL cell differentials. Cultured SSCS AMs exhibited a significant increase in O_2^- production; 2.4 (± 0.8) mmol $O_2^-/10^6$ AMs, compared with the control AM value of 0.4 (± 0.02) nmol $O_2^-/10^6$ AMs after incubation for 18 h with 10 µg/ml LPS (table 2).

Table 1. BAL eicosanoid concentration

Eicosanoid	BAL fluid, pg/ml	
	SSCS	control
LTE_4	742 (285)*	76 (2)
LTB_4	333 (108)	149 (24)
LTC_4	396 (155)	131 (21)
TxB_2	276 (20)	284 (15)
PGE_2	73 (1)	72 (1)
6-keto-$PGF_{1\alpha}$	818 (188)	457 (46)

All values are expressed as mean (± SEM). * $p < 0.05$.

Table 2. O_2^- production by BAL AMs

Condition	O_2^-, nmol/10^6 AMs	
	control	SSCS
Medium	0.4 (0.2)	1.6 (0.9)
Medium + PMA	2.4 (0.9)	6.7 (4.2)
LPS	0.4 (0.2)	2.4 (0.8)*
LPS + PMA	1.4 (0.4)	2.2 (0.9)

All values are expressed as mean (± SEM). * $p < 0.05$.

Light microscopy showed minor airway injury in the SSCS group that consisted of bronchial epithelial cell degeneration and desquamation. However, this airway injury was not consistent, since it was present in < 25% of the bronchi examined. In addition, there were focal clusters of neutrophils in perivascular and capillary spaces (fig. 1). Electron-microscopic examination demonstrated that the airway mucosa was infiltrated by eosinophils in the SSCS rabbits (fig. 2). No ultrastructural evidence of injury to the alveolar-capillary barrier in SSCS rabbits was observed. In the control rabbits, the light and electron micrographs showed no pathologic evidence of lung injury and only rare neutrophils and eosinophils.

The mean COHb% level after a standard SSCS exposure was 3.7 (± 0.2). This value is less than that found in asymptomatic human smokers of 5.1 (± 0.7) [13].

Discussion

Chronic exposure to SSCS caused lung injury that consisted of increases in pulmonary epithelial permeability, BAL LTE_4, and AM O_2^- production. In addition, SSCS caused minor airway injury characterized by infrequent epithelial cell degeneration and desquamation and histologic evidence of parenchymal injury as evidenced by focal clusters of neutrophils in the perivascular spaces.

The SSCS exposure regimen caused an increase in pulmonary epithelial permeability and a large increase in lung LTE_4 levels. Several investigators have postulated that ^{99m}TcDTPA molecules traverse the alveolar-capillary barrier through the epithelial layer [14, 15]. In a recent study, we showed that swelling of type I epithelium can possibly attenuate the passage of ^{99m}TcDTPA molecules through the alveolar-capillary barrier [16]. The electron micrographs from the SSCS group did not demonstrate any alterations in the alveolar-capillary barrier. Perhaps the small size of the ^{99m}TcDTPA molecules allows greater access through the sites of injury in the alveolar-capillary barrier. This injury site may be so small as to be virtually undetectable even with electron microscopy. Injury to the bronchial epithelial cells was demonstrated in scattered bronchi. However, this injury was not consistent, and it was considered that the bronchial clearance of ^{99m}TcDTPA constitutes a large enough portion of total pulmonary clearance to be responsible for the increase in ^{99m}TcDTPA lung uptake observed in the SSCS rabbits. Many investigators have demonstrated that sulfidopeptide leukotrienes, mainly LTC_4 and LTD_4, can increase vascular permeability [17–20]; but the possibility that LTE_4 can also cause increased permeability in alveolar epithelial cells cannot be dismissed.

Additionally, changes in lung volume and surfactant concentration have been shown to alter ^{99m}TcDTPA uptake [21–23], but lung surfactant concentration was not measured. Another possibility is that intact eosinophils can induce alveolar epithelial cell injury by the release of granule-associated products, possibly lysosomal enzymes [24]. Thus, eosinophils could increase pulmonary epithelial permeability by causing injury to lung alveolar epithelial cells.

In a previous paper [7], the acute effect of MS on rabbit lung eicosanoid metabolism was studied [7]. The MS exposure regimen consisted of 65 tidal volume breaths delivered via an ET over a period of 95 min. This acute exposure to large amounts of MS caused a significant increase in BAL 6-keto-$PGF_{1\alpha}$ and a decrease in BAL LTB_4. Also, 1 h after exposure to 60 tidal volume breaths

of diesel-fuel-polycarbonate plastic smoke, there was a significant decrease in BAL LTB_4 levels [16]. In contrast, in the present study, chronic exposure to 3 liters of SSCS mixed with air over a 15-min period/day for 20 days caused a significant increase in BAL LTE_4. However, lung LTB_4 levels did not change significantly after chronic exposure to SSCS.

Arachidonic acid, which is metabolized by the lipoxygenase pathway, is converted to an unstable intermediate, LTA_4, and then is either converted to LTB_4 or the sulfidopeptide leukotrienes (LTC_4, LTD_4, LTE_4) [25]. It is difficult to compare the eicosanoid data from these cigarette smoke studies due to differences in the type of cigarette smoke (MS vs. SSCS), dose of the smoke, and duration of exposure (acute vs. chronic). However, it is possible that these differences in the cigarette smoke exposure regimen may cause variable responses in the lipoxygenase pathway of arachidonic acid metabolism. Furthermore, other investigators have demonstrated that SSCS is not the same

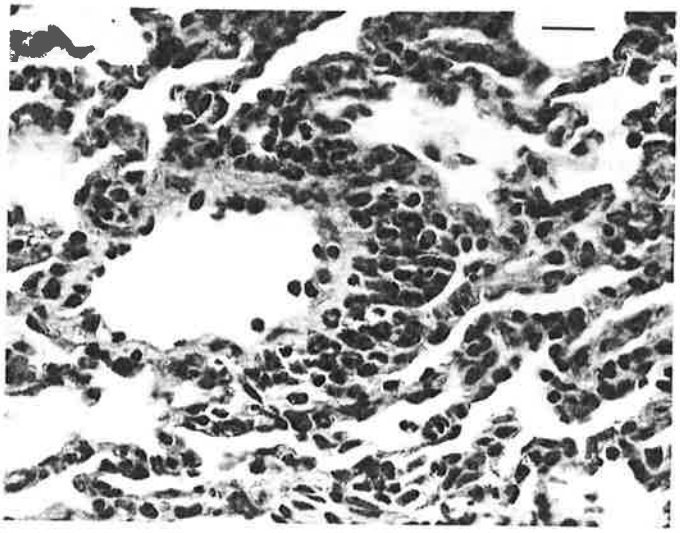

Fig. 1. Airway mucosa of an SSCS rabbit's lung parenchyma with evidence of neutrophils in perivascular and capillary spaces. HE. × 500. Bar = 18 μm.

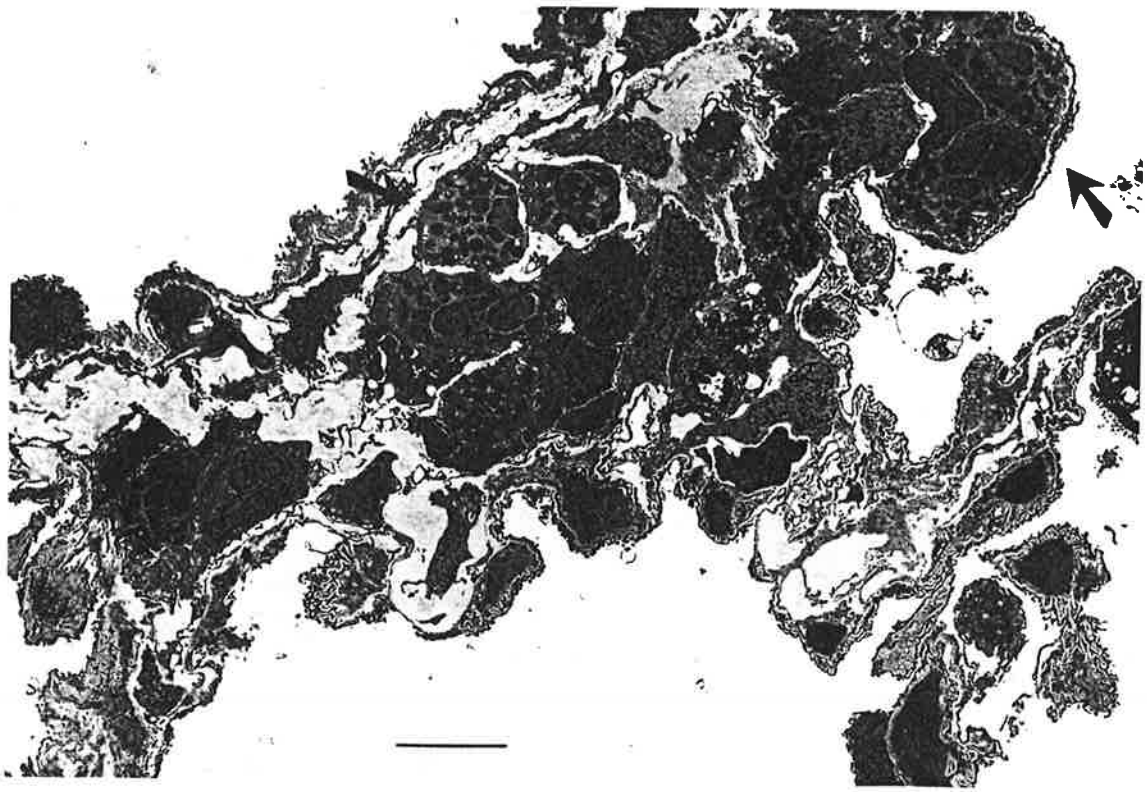

Fig. 2. Transmission electron micrograph of a SSCS rabbit's lung parenchyma with clusters of eosinophils (arrows). Uranyl acetate and lead citrate stain. × 1,200. Bar = 4 μm.

as MS in that SSCS has a greater carcinogenic effect on mouse skin [26] and it has different chemical characteristics [27].

Cultured SSCS AMs had significantly increased O_2^- production after activation with LPS (table 2). This finding suggests that SSCS-exposed AMs are more easily primed to spontaneously release oxygen species, possibly resulting in greater tissue injury. However, at this time, it is not possible to discern the relative roles of LTE_4 and/or the O_2^- radical in causing injury to alveolar epithelial cells that increased the pulmonary epithelial permeability of the SSCS-exposed rabbits.

In contrast to the LPS stimulation of O_2^- which was enhanced in the BAL AMs from SSCS rabbits, the response to PMA was not so stimulated (table 2). Failure of AMs to enhance their response to PMA after smoke exposure is not unique to this study but also occurred in rabbit AMs after exposure to smoke from burning diesel fuel with polycarbonate plastic where O_2^- production to PMA actually fell [28]. It is possible that acute diesel fuel smoke exposure damages the AM membrane so that the macrophage oxidase enzyme located in the membrane was not capable of utilizing NADPH as an electron source for the reduction of oxygen to the O_2^- radical [29]. Perhaps, chronic exposure to SSCS particulates, although of far less concentration than the diesel fuel smoke, affects the AM membrane in a similar manner, and, consequently, PMA administration in vitro does not increase AM O_2^- production. LPS may act through a different set of AM membrane receptors than does PMA.

The administration of PMA after the 18-hour LPS incubation period reduced O_2^- production, 41% for control AM and 67% for SSCS AM, compared to the 'medium + PMA' condition. Thus, it is concluded that PMA administration after LPS incubation is not an additive effect. Instead, prior LPS incubation appears to attenuate the ability of the AM to produce O_2^- in response to PMA. The intracellular level of Ca^{2+} appears to be very important in the ability of the AM to produce O_2^- by acting as a second messenger and causing the activation of Ca^{2+}-dependent protein kinases [29]. Thus, it is possible that LPS incubation may alter AM intracellular Ca^{2+} stores [30], and consequently the administration of PMA does not elicit further O_2^- production.

Other investigators have demonstrated that LPS exposure causes AMs to produce mRNA for Mn SOD [31]. Thus, another explanation for LPS incubation attenuating the ability of AMs to produce O_2^- after administration of PMA is that the AMs may have produced enough SOD to attenuate the PMA-induced increase in O_2^-. In the present study, the AMs were washed three times with PBS before PMA exposure, and we speculate our washings may have reduced the AM culture concentration of SOD. However, AM SOD concentrations were not measured in the present study, and the possibility that LPS incubation stimulated enough production of SOD to attenuate AM O_2^- levels after PMA administration cannot be dismissed.

The SSCS rabbits had a 5-fold increase in BAL total white cell count compared to controls. Perhaps, a period of 18 h after the last SSCS exposure is not a long enough recovery time from the acute effects of SSCS, and this may explain the increase in BAL white cell count. However, in a recent study, rabbits were exposed to JP-8 jet fuel vapors for 20 min/day at the same length of exposure as in the present study, 20 days. The JP-8 jet-fuel-exposed rabbits had a 44-fold increase in BAL total white cell counts compared to control values [32]. Furthermore, cultured JP-8 jet-fuel-exposed AMs were less adherent to plastic than control AMs and may be, consequently, more easily washed from the lungs. Thus, chronic SSCS exposure may cause an influx of inflammatory cells into the lungs and/or induce white cells to be less adherent and thus more easily washed from the lungs by BAL.

Gellert and coworkers [33] demonstrated that smokers had increased proportions of neutrophils and eosinophils in BAL fluid compared to nonsmokers [33]. In the present study, neutrophils were observed in the SSCS rabbits' perivascular and capillary spaces. Contrary to this observation, the SSCS BAL cell differentials were not different from that of the control rabbits and did not reflect our light- and electron-micrograph findings of neutrophilia and eosinophilia in the airways of SSCS rabbits. However, previous studies in our laboratory have demonstrated that BAL cell differentials do not necessarily reflect cell distribution obtained from histology [34]. Perhaps, the SSCS rabbits' short exposure regimen of 20 days did not allow enough time for the inflammatory cells to migrate into the alveolar septal areas. Another possibility is that the smoke exposure in our model is less than that experienced by an asymptomatic human smoker as evidenced by lower COHb levels [13]. Consequently, it is concluded that the smoke exposure is less in our model than for human smokers, and the amount of and toxicity of the smoke (MS vs. SSCS) may determine the nature and source of the inflammatory cell response.

Chronic SSCS caused minor airway injury as evidenced by scattered bronchial epithelial cell desquamation and degeneration as well as eosinophilia. One of the major symptoms of asthma in humans is airway hypersensitivity to aeroallergens. Perhaps, over the 20-day

SSCS exposure period, rabbits develop a similar hypersensitivity to SSCS. Another possibility is that chronic SSCS exposure increases lung permeability, and this allows more aeroallergens to invade the lung mucosa and contribute to the development of a chronic inflammatory state characterized by an influx of neutrophils and eosinophils.

It is concluded that the rabbit model has demonstrated that SSCS can cause lung injury. This lung injury is characterized by increased pulmonary epithelial permeability, changes in lung eicosanoid metabolism, increased AM O_2^- production, scattered histologic evidence of bronchial epithelial cell injury, and the influx of neutrophils and eosinophils into the lung mucosa.

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