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Acute neurogenic airway plasma exudation and edema induced by inhaled wood smoke in guinea pigs: role of tachykinins and hydroxyl radical

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Abstract

We studied the mechanisms underlying the wood smoke-induced acute airway injury in 120 anaesthetized guinea pigs. Five minutes after airway exposure, various doses of wood smoke produced a dose-dependent increase in Evans blue dye contents at all airway levels measured. Additionally, inhaled wood smoke produced submucosal edema of the trachea and bronchus, and peribronchial edema. These acute airway responses were nearly abolished by pretreatment with CP-96,345 alone [a tachykinin NK_1 receptor antagonist; (2*S*,3*S*)-cis-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)-methyl)-1-azabicyclo(2.2.2.)-octan-3-amine] or with a combination of CP-96,345 and dimethylthiourea (a hydroxyl radical scavenger), and were attenuated by pretreatment with dimethylthiourea alone, yet were not affected by pretreatment with SR-48,968 [a tachykinin NK_2 receptor antagonist; (*S*)-*N*-methyl-*N*(4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl)benzamide], with a combination of CP-96,344 and SR-48,965 (inactive enantiomers), with MK-886 [a leukotriene biosynthesis inhibitor; L-663,536(3-(1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl)-2,2-dimethylpropanoic acid], with indomethacin (a cyclooxygenase inhibitor), or with N^G-nitro-L-arginine methyl ester (a nitric oxide (NO) synthase inhibitor). The activity of airway neutral endopeptidase (an enzyme for tachykinin degradation) was not influenced by wood smoke at 5-min post-exposure. We conclude that both endogenous tachykinins and hydroxyl radical play an important role in producing smoke-induced acute airway plasma exudation and airway edema in guinea pigs. The contribution of tachykinins to these neurogenic responses is mediated via the activation of tachykinin NK_1 receptors and partly via a hydroxyl radical mechanism, and is not associated with inactivation of neutral endopeptidase.

Keywords: Wood smoke; CP-96,345; Tachykinin NK_1 receptor; Tachykinin NK_2 receptor

1. Introduction

Smoke inhalation injury is one of the most severe complications associated with fire-related disasters (Crapo, 1981). Inhalation of toxic smoke is known to initially cause acute airway injury in humans (Traber and Herndon, 1986) and laboratory animals (Hales et al., 1989; Barrow et al., 1990; Traber et al., 1992; LaLonde et al., 1994; Sakurai et al., 1998). Among several symptoms, airway microvascular leakage and airway edema are two cardinal signs of smoke-induced airway injury (Traber and Herndon, 1986; Traber et al., 1992; Barrow et al., 1990). Plasma exudation resulting from microvascular leakage is an important component of airway inflammation (Barnes, 1996a). Tissue edema may possibly obstruct the airways and contribute to ventilation–perfusion mismatching, respiratory insufficiency, and lung atelectasis observed in fire victims (Crapo, 1981; Traber and Herndon, 1986). While these two smoke-induced airway responses are well recognized, their pathophysiological mechanisms remain largely unclear.

Tachykinins, such as substance P and neurokinin A, are proinflammatory sensory neuropeptides released locally from airway afferent C-fibre nerve endings in response to various chemical irritants (Solway and Leff, 1991; Barnes, 1996a). Once released, tachykinins can cause several neurogenic airway responses including plasma exudation and tissue edema (Solway and Leff, 1991; Barnes, 1996a; Advenier et al., 1997). Their effects are mediated mainly via activation of tachykinin NK_1 and NK_2 receptors (Sol-

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way and Leff, 1991; Barnes, 1996a; Advenier et al., 1997). The tachykinins released are rapidly degraded by neutral endopeptidase, and inhibition of this enzyme may exaggerate the airway effects of tachykinins (Solway and Leff, 1991; Barnes, 1996a). We previously reported that inhaled wood smoke stimulates lung vagal C-fibre afferent nerve endings (Lai and Kou, 1998) and inactivates airway neutral endopeptidase (Hsu et al., 1998a, 2000). This raises the possibility that tachykinin mechanisms may participate in smoke-induced airway responses. Indeed, endogenous tachykinins have been shown to be responsible for producing acute bronchoconstriction (Hsu et al., 1998b) and airway hyperreactivity (Hsu et al., 1998a) following wood smoke inhalation. However, the importance of tachykinins and their degradation enzyme in the pathogenesis of smoke-induced acute airway plasma exudation and airway edema has not been elucidated.

In addition to tachykinins, inhalation of toxic smoke may elevate the oxygen radical burden in the airways (Pryor, 1992; Youn et al., 1992), increase the release of other chemical mediators such as lipoxygenase (i.e. leukotrienes) and cyclooxygenase products (i.e. prostaglandins and thromboxane) (Traber and Herndon, 1986; Quinn et al., 1990), and activate nitric oxide (NO) synthase to generate NO (Soejima et al., 1999). Among the major types of oxygen radicals, the hydroxyl radical (·OH) is extremely reactive and NO has many biological functions. Lipoxygenase and cyclooxygenase products are arachidonate metabolites. These two categories of chemical mediators are known to possess potent effects on the production of airway plasma exudation and airway edema (Chung et al., 1990; Barnes, 1996b; Barnes et al., 1998; Lei et al., 1996), and have been implicated in the pathogenesis of inhalation injury (Quinn et al., 1990; Youn et al., 1992; Loick et al., 1993; Soejima et al., 1999). Nevertheless, the role of ·OH, arachidonate metabolites, and NO in the development of smoke-induced acute airway plasma exudation and airway edema is not completely understood.

The present study with anaesthetized guinea pigs was undertaken to determine (1) the dose–response relationship and time course of acute airway plasma exudation produced by inhaled wood smoke, (2) the relative contributions of tachykinins, ·OH, arachidonate metabolites, and NO to smoke-induced airway exudative and edematous responses, and (3) whether these acute airway responses are associated with a smoke-induced reduction in airway neutral endopeptidase activity.

2. Materials and methods

2.1. Animal preparation

Male Hartley guinea pigs (body weight, 307 ± 4 g; $n = 120$) were anaesthetized with an intraperitoneal injection of chloralose (100 mg/kg; Sigma, St. Louis, MO, USA) and urethane (500 mg/kg; Sigma). The carotid artery and jugular vein were cannulated for recording arterial blood pressure and for intravenous administration of pharmacological agents, respectively. During the course of the experiments, supplemental doses of chloralose (20 mg/kg/h) and urethane (100 mg/kg/h) were administered to maintain the abolition of pain reflexes induced by pinching the animal's hindpaw. The animals were fixed in a supine position and the trachea was cannulated below the larynx with a short tracheal cannula via a tracheotomy, through which the animals were ventilated with a rodent respirator (model 683, Harvard Apparatus, South Natick, MA, USA) at a constant rate of 60 breaths/min. Tidal volume was adjusted according to the body weight of each animal (10 ml/kg) and was kept constant in each experiment. The animals were then paralyzed with an intravenous injection of pancuronium bromide (0.1 mg/kg/h; Organon Teknika, Boxtel, Holland). Throughout the experiment, the body temperature of the animals was maintained at ~ 36°C by means of a servo heating blanket. All protocols were in accordance with the guidelines for the care and use of laboratory animals published by the National Institutes of Health, Bethesda, MD, USA and were approved by the Committee of the National Science Council, Taipei, Taiwan.

2.2. Generation and delivery of wood smoke

The electric furnace and the methods for generating wood smoke were described in detail in our previous report (Kou and Lai, 1994). In brief, 100 g of dry wood dust (lauan wood) was thermally decomposed in a furnace (model 101, Nan Jou, Taipei, Taiwan) at a core temperature maintained at 500 ± 8°C for 5 min and the effluent smoke was collected in a 25-l plastic balloon attached to the furnace outlet. Wood smoke generated by this method contains approximately 1.5% O_2, 15% CO_2, 24% CO, and 25 mg/l particulates (Kou et al., 1995). Immediately after smoke generation, the plastic balloon containing the fresh smoke was attached to the inspiratory inlet of the respirator via a 3-way stopcock. Wood smoke at a temperature of ~ 25°C was delivered into the lungs when the 3-way stopcock was turned to connect the respirator with the balloon. Before each smoke challenge, the lungs were hyperinflated ($4 \times$ tidal volume) to establish a constant volume history. To avoid contamination, the expired smoke was drawn into a fume hood via a suction line.

2.3. Measurements of plasma exudation

The magnitude of plasma exudation was quantified by measuring the extravasation of Evans blue dye, using a method described previously (Evans et al., 1988). Evans blue dye (25 mg/kg) was injected intravenously 1 min

before the air or smoke challenge. Five, 60, or 120 min after airway challenge, the thorax was quickly opened and the lungs were inflated, using a pressure of 5 cm H_2O. Both the right and left atria were incised. A blunt, 12-gauge needle was inserted into the pulmonary artery via a ventriculotomy. The heart was clamped and the pulmonary circulation was perfused with 25 ml of saline at a pressure of 30 mm Hg in order to remove the intravascular dye. Subsequently, the left ventricle was opened and the systemic circulation was perfused with 200 ml of saline at a pressure of 120 mm Hg. Following vascular perfusion, the left bronchus was ligated and the trachea was cut longitudinally. The airways and lungs were then excised and separated into left and right portions; the left portion was immediately processed for histological preparations. The connective tissues, vasculature, and parenchyma of the right lung were gently scraped off with a blunt scalpel until only the airway tissue was left. The right airway tissues were then sectioned and subdivided into three levels: the trachea, main bronchus, and intrapulmonary airways. Both the airway and scraped parenchyma tissues were blotted dry and weighed. Tissue Evans blue dye was extracted by incubation in 2 ml of formamide (Sigma) at 40°C for 24 h and its concentration was measured by light absorbance (Multiscan spectrophotometer, U-100, Hitachi, Japan) at 620 nm. The tissue content of Evans blue dye was calculated by interpolation on a standard curve of dye concentrations in the range of 0.05–10 μg/ml and was expressed as ng dye/mg of wet tissue.

2.4. Histological preparations and examinations

Immediately after their excision, the left portions of the airway and lung tissues were fixed by immersion in a buffered neutral formalin solution for 48 h. Tissue specimens were embedded in paraffin and were cut transversely into 5 μm thick sections which were subsequently stained with hematoxylin and eosin. For each animal, one histological section was obtained from the trachea and also from the main bronchus, while two sections were obtained from the lung tissues. These sections were examined and photographed with a light microscope (Axioskop MC 100, Zeiss, Germany) at a magnification of 200× or 400×. The degree of submucosal edema in the trachea and bronchus was determined from the ratio of the submucosal layer thickness to the epithelial thickness (submucosal edema index). The degree of peribronchial edema was estimated by a method modified from that reported previously (Garcia et al., 1994). In brief, photographs of histological sections were superimposed with a transparency comprising 3620 points in 605 cm^2. Points falling on the peribronchial cuff of the connective tissue were counted and were considered as an index of the area occupied by interstitial edema. The number of points falling on the peribronchial cuff was divided by the perimeter of the corresponding airway to give an index of tissue edema. This procedure was done in order to avoid possible artifacts dependent on differences in airway size. For each histological section, structures at three different areas were randomly selected and their degrees of tissue edema were averaged.

2.5. Determination of airway neutral endopeptidase activity

The activity of airway neutral endopeptidase was determined as described previously (Hsu et al., 1998a). Briefly, frozen airway tissues were thawed and minced in tubes containing 50 mM Tris, pH 7.4. The tissues were then sonicated for 30 s at 4°C and centrifuged at 17,500 × *g* for 15 min. The supernatant was removed for analysis. In the presence of substrate, the amount of 2-naphthylamine released by the tissues was determined using a spectrophotometer (U-100, Hitachi) at 530 nm. The phosphoramidon-inhibitable neutral endopeptidase specific activity was expressed as nmol/mg of protein/h.

2.6. Pharmacological pretreatments

A tachykinin NK_1 receptor antagonist [CP-96,345, 5 mg/kg, 5 mg/ml; (2*S*,3*S*)-cis-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)-methyl)-1-azabicyclo(2.2.2.)-octan-3-amine; Pfizer, Groton, CT, USA], an inactive enantiomer of CP-96,345 [CP-96,344, 5 mg/kg, 5 mg/ml; (2*R*,3*R*)-cis-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)-methyl)-1-azabicyclo(2.2.2.)-octan-3-amine; Pfizer], a tachykinin NK_2 receptor antagonist [SR-48,968, 2 mg/kg, 1 mg/ml; (*S*)-*N*-methyl-*N*(4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl)benzamide; Sanofi Research, Montpellier, France], and an enantiomer of SR-48,968 [SR-48,965, 2 mg/kg, 1 mg/ml; (*R*)-*N*-methyl-*N*(4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl)benzamide; Sanofi Research] were each dissolved in isotonic saline. A NO synthase inhibitor (L-NAME, 10 mg/kg; N^G-nitro-L-arginine methyl ester; Sigma) was also dissolved in isotonic saline to a concentration of 10 mg/ml. A leukotriene biosynthesis inhibitor [MK-886, 10 mg/kg; L-663,536(3-(1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl)-2,2-dimethylpropanoic acid); Calbiochem, CA, USA] was first dissolved in 200 μl dimethyl sulfoxide (Sigma) and then diluted with saline to a final concentration of 10 mg/ml. A cyclooxygenase inhibitor (indomethacin, 10 mg/kg; Sigma) was first dissolved in polyethylene glycol and then diluted in a 1:1 ratio with saline to a final concentration of 10 mg/ml. All the above-mentioned drugs were injected as boluses into the vein. A ·OH scavenger (dimethylthiourea, 4.5 g/kg; Sigma) was dissolved in isotonic saline to a concentration of 0.75 g/ml and was slowly infused into the vein for a period of 20 min with an infusion pump (Sage 367, Cambridge, MA, USA). We used two criteria to judge whether the doses of compounds used were sufficient

enough. First, we looked for the doses of these drugs that are equal to or higher than those previously used by other investigators (Evans et al., 1988; Guhlmann et al., 1989; Sakamoto et al., 1994; Lei et al., 1996; Kageyama et al., 1997) in studies of lung pathophysiology induced by other experimental conditions in guinea pigs. Second, in the preliminary study, we doubled or tripled the dose if any compound failed to influence the smoke-induced airway responses.

2.7. Experimental procedures

A total of 120 animals were randomly and equally divided into 20 groups. In Study 1, airway exudative responses to air and to 10, 20, or 40 breaths of wood smoke were investigated at 5-min post-smoke exposure in four groups of animals. Additionally, airway exudative responses to air and to 40 breaths of wood smoke were investigated at 60- or 120-min post-smoke exposure in another four groups of animals. In Study 2, airway exudative and edematous responses to air or 40 breaths of wood smoke were investigated at 5-min post-smoke exposure in two groups of animals to establish the air/vehicle and smoke/vehicle controls, respectively. Additionally, airway exudative and edematous responses to 40 breaths of wood smoke were investigated at 5-min post-smoke exposure in eight groups of animals pretreated with either CP-96,345 alone, SR-48,968 alone, dimethylthiourea alone, CP-96,345 and dimethylthiourea in combination, CP-96,344 and SR-48,965 in combination, MK-886, indomethacin, or L-NAME 10–15 min before smoke challenge. In Study 3, one group of animals received a sham air challenge, while the other group received 40 breaths of wood smoke. Five minutes after the air or smoke exposure, the animals were killed by an overdose of anaesthetics. Then the airway tissues including the tracheal segment below the tip of the tracheal cannula and the main stem bronchus were quickly removed, washed with isotonic saline, and stored at −70°C for analysis of neutral endopeptidase activity.

2.8. Statistical analysis

Results of Evans blue dye extravasation, submucosal edema index, and peribronchial edema index were analyzed by a one-factor analysis of variance followed by Fisher's least significant difference procedure when appropriate. Data for airway neutral endopeptidase activity were analyzed with Student's *t*-test. $P < 0.05$ was considered significant. All data are presented as means ± S.E.

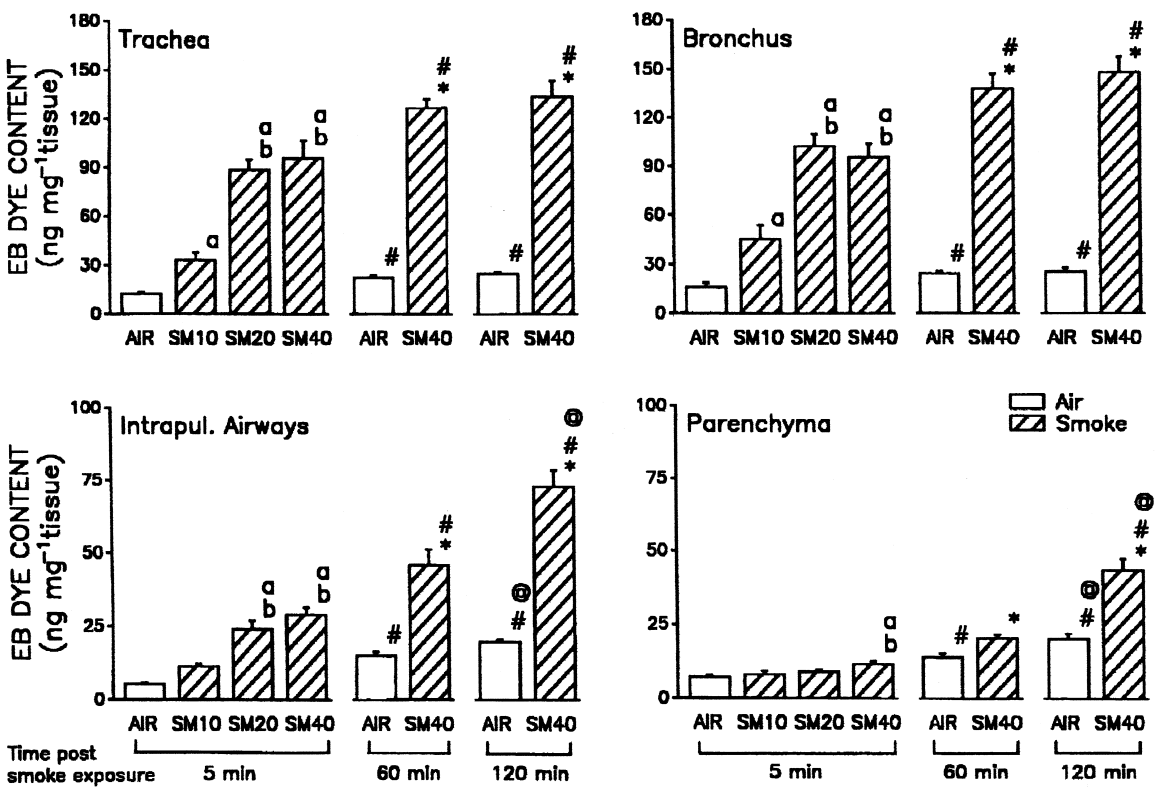

Fig. 1. Evans blue (EB) dye extravasation of trachea, bronchus, intrapulmonary airways, and lung parenchyma induced by air or various doses of wood smoke measured at different times (5, 60, and 120 min) post-smoke exposure. SM10, SM20, and SM40 represent 10, 20, and 40 breaths of smoke, respectively. [a] significantly different from response to air at 5 min; [b] significantly different from response to SM10 at 5 min; * significantly different from the corresponding response to air at 60 or 120 min; # significantly different from the corresponding response to air or SM40 at 5 min; @ significantly different from the corresponding response to air or SM40 at 60 min. Data in each group are means ± S.E. for six animals.

3. Results

3.1. Dose–response relationship and time course of airway exudative response

Five-minute post-airway exposure and then after vascular perfusion, wood smoke acutely and markedly produced airway plasma exudation at all airway levels measured, as evidenced by an increase in Evans blue dye content in these tissues (Fig. 1). When various tidal breaths of wood smoke were delivered, the airway exudative responses were dose-dependent. Statistical analysis revealed that delivery of 20 or 40 breaths of wood smoke significantly increased dye contents at all airway levels, and that delivery of 10 breaths of wood smoke significantly increased dye contents in the trachea and bronchus (Fig. 1). Furthermore, there was no significant difference between the airway exudative responses induced by 20 and by 40 breaths of wood smoke, but both were significantly greater than the response induced by 10 breaths of wood smoke (Fig. 1). In contrast, only delivery of up to 40 breaths of wood smoke induced a small but significant increase in dye content in lung parenchyma (Fig. 1). When the time of vascular perfusion was delayed to either 60- or 120-min post-smoke exposure, the exudative responses to 40 breaths of wood smoke at all airway levels and in lung parenchyma still persisted and were greater than those measured at 5-min post-airway exposure (Fig. 1). The responses to 40 breaths of wood smoke with vascular perfusion 5-min post-airway exposure were then taken as the standard responses for subsequent studies in animals pretreated with various pharmacological agents or a saline vehicle.

3.2. Effects of pharmacological pretreatments on airway exudative and edematous responses

In response to delivery of 40 breaths of wood smoke, the airways of animals pretreated with saline vehicle fully displayed Evans blue dye extravasation (Fig. 2). Additionally, histological examinations revealed substantial submucosal edema in the trachea (Fig. 3B1) and bronchus (Fig. 3B2), and apparent peribronchial edema (Fig. 3B3). However, neither major morphological changes in the alveolar region nor alveolar flooding were noted. In animals pretreated with CP-96,345 alone, dimethylthiourea alone, or CP-96,345 and dimethylthiourea in combination, both the airway exudative (Fig. 2) and edematous responses (Fig. 4) to inhaled wood smoke were significantly attenuated. In sharp contrast, in animals pretreated with SR-48,968 alone,

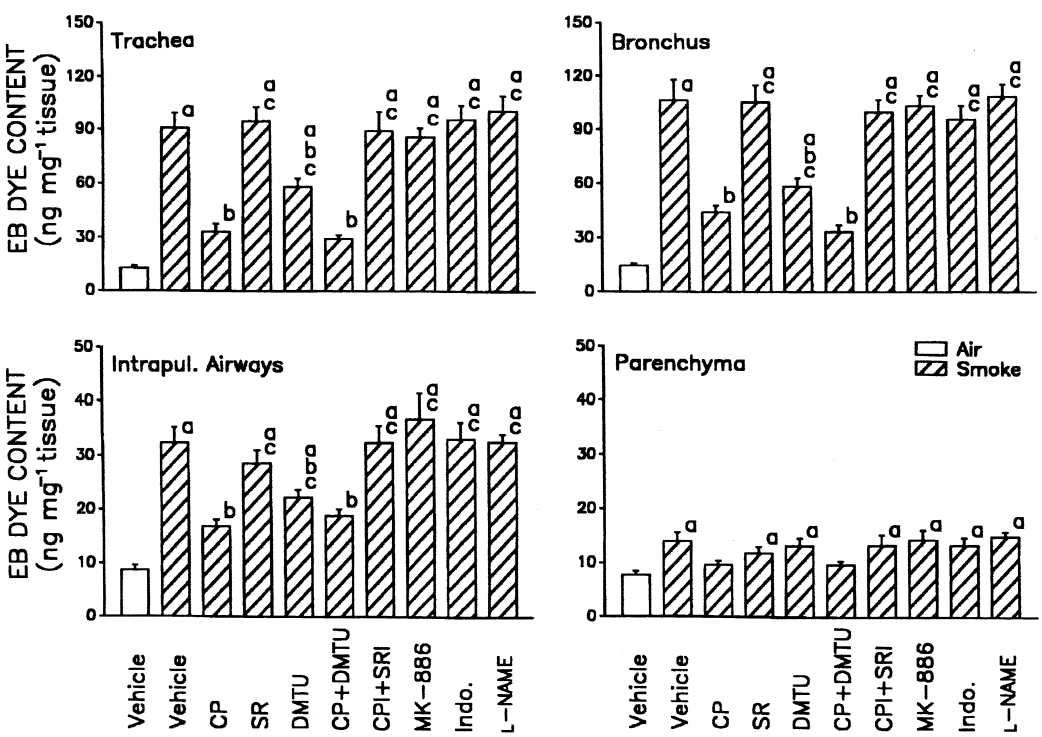

Fig. 2. Evans blue (EB) dye extravasation of trachea, bronchus, intrapulmonary airways, and lung parenchyma induced by air or 40 breaths of wood smoke measured at 5 min post-smoke exposure in animals pretreated with saline vehicle, CP-96,345 alone (CP; 5 mg/kg), SR-48,968 alone (SR; 2 mg/kg), dimethylthiourea alone (DMTU; 4.5 g/kg), CP-96,345 (5 mg/kg) and DMTU (4.5 g/kg) in combination (CP + DMTU), CP-96,344 (5 mg/kg) and SR-48,965 (2 mg/kg) in combination (CPI + SRI), MK-886 (10 mg/kg), indomethacin (Indo.; 10 mg/kg), or L-NAME (10 mg/kg). [a] significantly different from response to air in animals pretreated with saline vehicle; [b] significantly different from response to smoke in animals pretreated with saline vehicle; [c] significantly different from response to smoke in animals pretreated with CP. Data in each group are means ± S.E. for six animals.

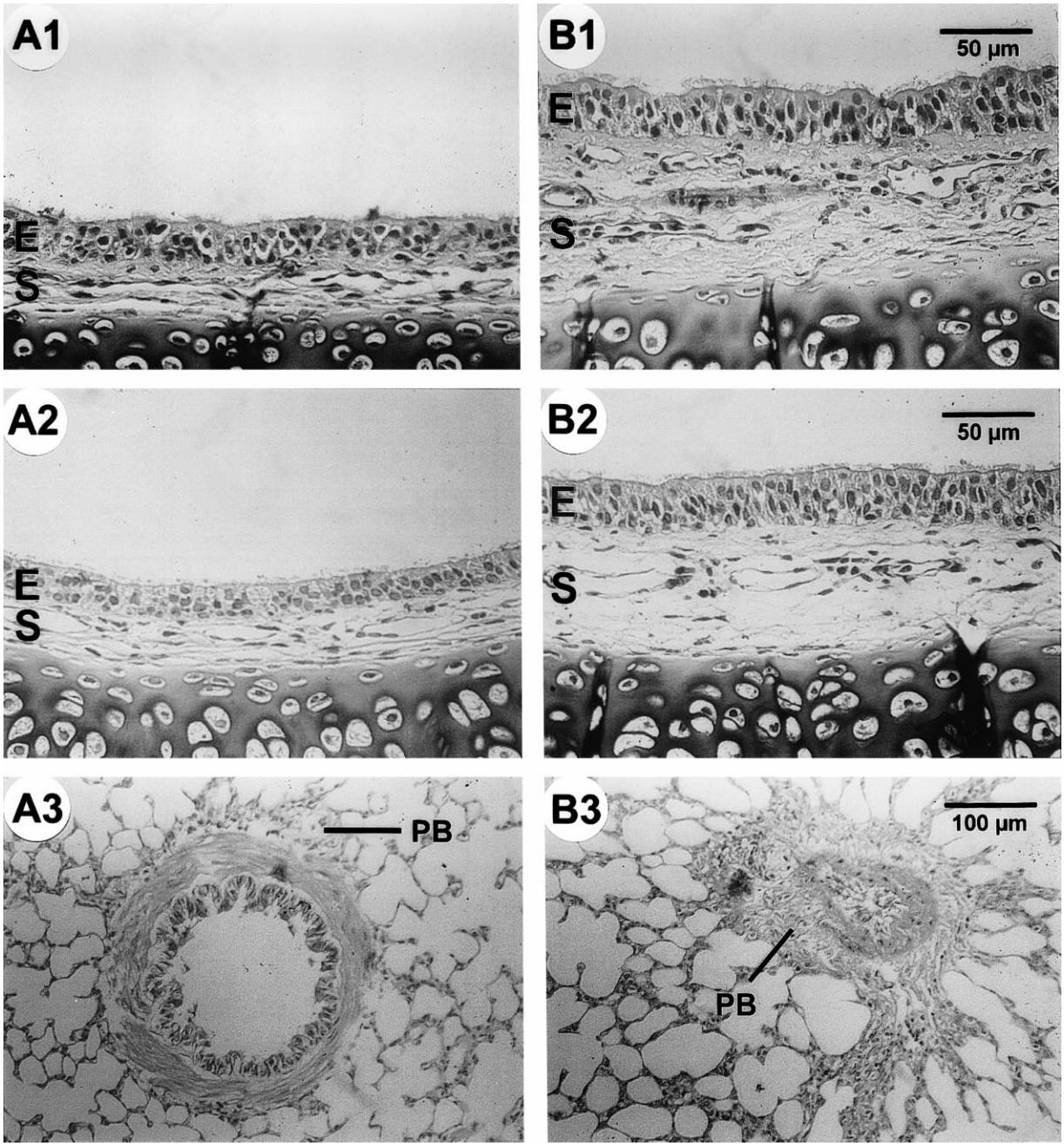

Fig. 3. Light micrographs of epithelial and submucosal space of trachea (A1 and B1) and bronchus (A2 and B2), and peribronchial cuff (A3 and B3) in two guinea pigs. Tissues were excised 5 min post-airway exposure to air (A1, A2, and A3) or 40 breaths of wood smoke (B1, B2, and B3). E, epithelium; S, submucosal space; PB, peribronchial cuff. Magnification: 400× in A1, A2, B2, and B2; 200× in A3 and B3. Note that both submucosal space and peribronchial cuff were greatly enlarged in tissues exposed to smoke.

CP-96,344 and SR-48,965 in combination, MK-886, indomethacin, or L-NAME, these smoke-induced airway responses were not significantly affected (Figs. 2 and 4). Furthermore, smoke-induced exudative responses at all airway levels (Fig. 2) and submucosal edema of the trachea and bronchus (Fig. 4) were moderately reduced by dimethylthiourea to magnitudes that were still significantly different from the air controls, and were greatly reduced by CP-96,345 to magnitudes that were not significantly different from the air controls (Fig. 2). Pretreatment with CP-96,345 and dimethylthiourea in combination did not further attenuate either of the smoke-induced airway responses, as compared to pretreatment with CP-96,345 alone (Figs. 2 and 4). Finally, the small but significant increase in Evans blue dye content in lung parenchyma was also abolished by pretreatment with CP-96,345 alone or in combination with dimethylthiourea, but was not affected by other pharmacological interventions (Fig. 2).

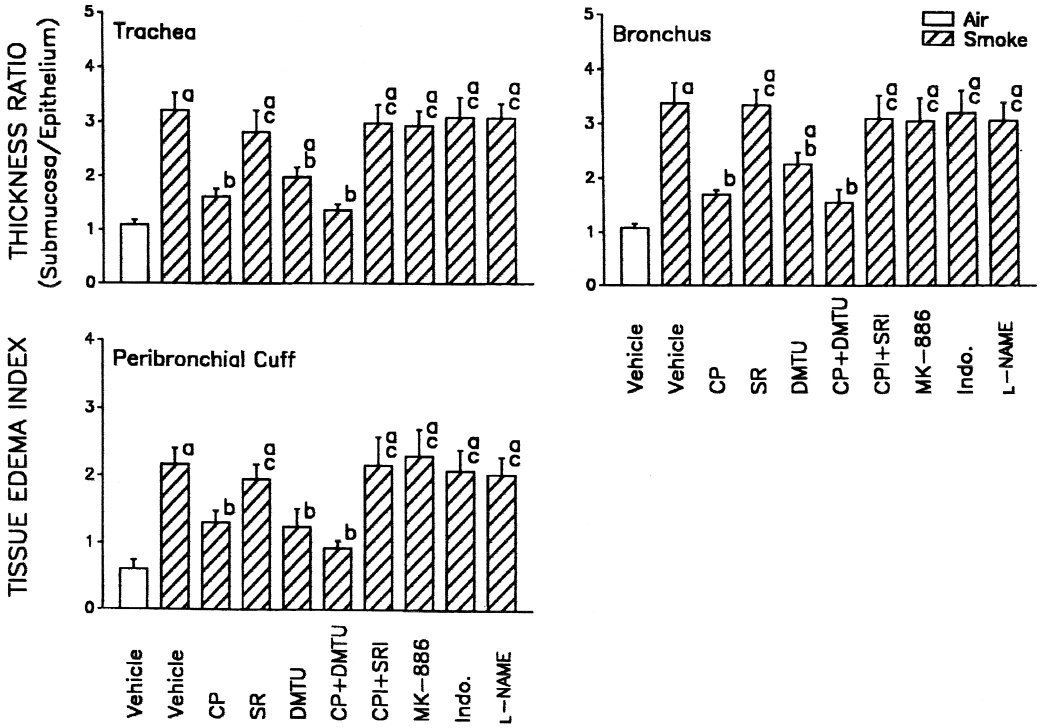

Fig. 4. Increases in ratio of submucosal thickness to epithelial thickness, and peribronchial edema index induced by air or 40 breaths of wood smoke in animals pretreated with saline vehicle, CP-96,345 alone (CP; 5 mg/kg), SR-48,968 alone (SR; 2 mg/kg), dimethylthiourea alone (DMTU; 4.5 g/kg), CP-96,345 (5 mg/kg) and DMTU (4.5 g/kg) in combination (CP + DMTU), CP-96,344 (5 mg/kg) and SR-48,965 (2 mg/kg) in combination (CPI + SRI), MK-886 (10 mg/kg) , indomethacin (Indo.; 10 mg/kg), or L-NAME (10 mg/kg). [a] significantly different from response to air in animal pretreated with saline vehicle; [b] significantly different from response to smoke in animals pretreated with saline vehicle; [c] significantly different from response to smoke in animals pretreated with CP. Data in each group are means ± S.E. for six animals. See text for details of measurement of peribronchial edema index.

3.3. Effects of wood smoke on airway neutral endopeptidase activity

The activity of airway neutral endopeptidase measured in tissues excised 5 min after delivery of 40 breaths of wood smoke (47.2 ± 8.4 nmol/mg of protein/h) was not significantly different from that in tissues excised at the same time after sham air challenge (45.2 ± 4.2 nmol/mg of protein/h; $P > 0.05$, $n = 6$).

4. Discussion

We demonstrated that, in anaesthetized guinea pigs, delivery of wood smoke acutely induced airway plasma exudation and airway edema. The former response, lasting for > 2 h, was evidenced by a large dose-dependent increase in Evans blue dye content in tissues and may have resulted from an increase in airway microvascular permeability. The latter response was revealed histologically by an enlargement of the submucosal space and peribronchial cuff and was probably due to the accumulation of exudative fluid and macromolecules within airway tissues. In contrast, only delivery of the largest dose of wood smoke produced a slight increase in dye content in lung parenchyma and caused no obvious histological changes in the alveolar region or alveolar flooding. These effects are similar to those reported from clinical and animal studies (Moylan et al., 1972; Traber and Herndon, 1986; Barrow et al., 1990; Sakurai et al., 1998), in which airway injury was prominent within the first few hours after toxic smoke exposure, while lung parenchyma edema occurred with a much longer delay from several hours to days after inhalation injury. Furthermore, the present study showed that dramatic pathophysiological changes in the airways occur as rapidly as a few minutes after smoke inhalation. In addition to the dose-dependent relationship of the smoke-induced increase in dye content in tissues, delivery of 10 breaths of wood smoke significantly increased dye contents only in the trachea and bronchus, whereas delivery of 20 or 40 breaths of wood smoke significantly increased dye contents at all airway levels. Furthermore, only delivery of up to 40 breaths of wood smoke induced a significant increase in dye content in lung parenchyma. Therefore, it appears that a greater amount of wood smoke may damage a greater area of airway tissues, from the large airways to the lung parenchyma.

In this study, pretreatment with CP-96,345 (a tachykinin NK_1 receptor antagonist) nearly abolished both the airway exudative and edematous responses to inhaled wood smoke,

whereas pretreatment with either SR-48,968 (a tachykinin NK_2 receptor antagonist) or a combination of CP-96,344 and SR-48,965 (inactive enantiomers) failed to do so. These results suggest that endogenous tachykinins play an essential role in the production of smoke-induced acute airway plasma exudation and airway edema, and that the contribution of tachykinins is primarily mediated through the activation of tachykinin NK_1 receptors. Neurogenic airway responses, involving C-fibre afferents and their containing tachykinins, have been well documented as major consequences of airway assault by various inhaled irritants such as cigarette smoke or ozone (Solway and Leff, 1991; Barnes, 1996a; Lei et al., 1996). In a recent study (Hsu et al., 1998b), we found that the activation of tachykinin NK_2 receptors by endogenous tachykinins is responsible for producing the bronchoconstriction induced by inhaled wood smoke. Thus, our observations are in good agreement with the current viewpoint regarding neurogenic airway responses, that plasma extravasation and airway smooth muscle contraction are predominantly mediated through the activation of tachykinin NK_1 and NK_2 receptors, respectively (Solway and Leff, 1991; Barnes, 1996a; Advenier et al., 1997). The contribution of tachykinins to the observed airway exudative and edematous responses may originate from an increase in their release from airway afferent C-fibre nerve endings when wood smoke stimulates these pulmonary receptors (Kou et al., 1995; Lai and Kou, 1998) and/or from smoke-induced inactivation of airway neutral endopeptidase, the major enzyme for tachykinin degradation (Solway and Leff, 1991; Barnes, 1996a). We previously reported that the activity of airway neutral endopeptidase in guinea pigs is reduced 15 min after inhalation of 15 breaths of wood smoke (Hsu et al., 1998a, 2000). However, we found no evidence of the involvement of neutral endopeptidase inactivation in the acute airway exudative and edematous responses observed in the present study, because neutral endopeptidase activity was not significantly affected in airway tissues excised 5 min after delivery of 40 breaths of wood smoke, a time when these airway responses occurred. In a follow-up study, we found that the activity of airway neutral endopeptidase in guinea pigs was reduced 120 min after inhalation of 40 breaths of wood smoke (Y.S. Lin and Y.R. Kou, unpublished data). Therefore, it would be assumed that the time after smoke insult, but not the amount of wood smoke, could be a more important factor for the inactivation of airway neutral endopeptidase by inhaled wood smoke.

We further demonstrated that pretreatment with dimethylthiourea, an effective ·OH scavenger, moderately but significantly attenuated both the airway exudative and edematous responses to inhaled wood smoke, suggesting that ·OH is actively involved in producing these acute airway responses. Several investigators have shown that, in a sheep model, airway and/or lung injury assessed several hours after toxic smoke inhalation can be ameliorated by administration of a scavenger for oxygen radicals (Kimura et al., 1988), an enzyme that metabolizes oxygen radicals (Nguyen et al., 1995), or an iron chelator that suppresses the production of oxygen radicals (LaLonde et al., 1994). The present findings indicate that oxygen radicals actually participate in the very early process of inhalation injury. Regardless of the time course of their participation, there is little doubt that oxygen radicals play a contributory role in the pathogenesis of smoke inhalation injury (Pryor, 1992). Likewise, oxygen radicals have also been largely implicated in airway and/or lung injury induced by various oxidant irritants such as cigarette smoke and ozone (Pryor, 1992; Youn et al., 1992; Lei et al., 1996). The source of the ·OH that produces the acute airway responses observed in this study remains unclear, but wood smoke may be one possible origin. The gas phase of wood smoke is known to contain high concentrations of free radicals and radical precursors which are formed during combustion (Pryor, 1992). These free radicals and their precursors may continuously generate oxygen radicals either in the smoke or upon reaching the airways (Traber and Herndon, 1986; Pryor, 1992; LaLonde et al., 1994). The other possible origin is that ·OH may be formed and released endogenously by certain inflammatory cells when they are activated by smoke inhalation (Traber and Herndon, 1986). Indeed, there is substantial evidence that oxygen radical activity is increased in airways and lungs exposed to toxic smoke (Demling et al., 1994, 1995).

Despite the fact that their contributions are clear, the exact mechanisms by which tachykinins and ·OH participate in the development of smoke-induced acute airway plasma exudation and airway edema remain unclear. Both tachykinins and ·OH are known to have direct injurious effects on the microvasculature (Rubanyi, 1988; Chung et al., 1990; Barnes, 1996a). Furthermore, tachykinins, acting as vasodilators, may also increase bronchial blood flow (Solway and Leff, 1991; Barnes, 1996a), which may facilitate airway plasma exudation (Chung et al., 1990). In fact, smoke-induced acute airway injury is usually accompanied by a large increase in bronchial blood flow (Kramer et al., 1989; Traber et al., 1992). Therefore, tachykinins and ·OH might act independently in different airway tissues to produce these two airway responses. However, in the present study, pretreatment with CP-96,345 and dimethylthiourea in combination did not seem to further attenuate these two airway responses, as compared to pretreatment with CP-96,345 alone. Accordingly, it appears that there is an overlap of functional contributions of tachykinins and ·OH to smoke-induced airway exudative and edematous responses. ·OH may inhibit airway neutral endopeptidase and exaggerate the airway effects of tachykinins (Solway and Leff, 1991; Barnes, 1996a). This is unlikely, however, because airway neutral endopeptidase was not inhibited in this study. A plausible explanation for the overlap of contributions is that tachykinins and ·OH are interrelated in their release. For example, it has been shown that ·OH

is the major chemical factor responsible for the stimulation of bronchopulmonary C-fibre afferents by inhaled wood smoke (Kou et al., 1997; Lai and Kou, 1998). Thus, scavenging ·OH by dimethylthiourea would reduce both the magnitude of the stimulation of C-fibre nerve endings by wood smoke and the amount of the subsequent release of tachykinins available for producing these two airway responses. However, dimethylthiourea presumably could not interfere with the part of the responses caused by the release of tachykinins due to smoke-related stimuli other than ·OH. Additionally, it has been demonstrated that tachykinins have the ability to increase the production of oxygen radicals from certain lung cells (Brunelleschi et al., 1990; Murris-Espin et al., 1995). In this case, blocking tachykinin receptors by their antagonist may possibly eliminate the contributions from both tachykinins and ·OH.

Inhaled toxic smoke can increase the release of cyclooxygenase and lipoxygenase products (Traber and Herndon, 1986; Quinn et al., 1990), and activate NO synthase to generate NO (Soejima et al., 1999). It is known that these arachidonate metabolites and NO can increase airway microvascular permeability (Chung et al., 1990; Barnes, 1996b; Barnes et al., 1998). However, pretreatment with either indomethacin (a cyclooxygenase inhibitor), MK-886 (a leukotriene biosynthesis inhibitor), or L-NAME (a NO synthase inhibitor) failed to modify the acute airway exudative and edematous responses to wood smoke, suggesting that these arachidonate products and NO are unlikely to play a vital role. It has been shown that pulmonary edema observed 6 h after smoke inhalation in rats (Shinozawa et al., 1986) and 3 h after smoke inhalation in sheep (Quinn et al., 1990) can be attenuated by pretreatment with a cyclooxygenase inhibitor and a lipoxygenase inhibitor, respectively. Additionally, it has been reported that the increase in airway blood flow and the deterioration of arterial blood oxygenation observed 48 h after smoke inhalation in sheep can be attenuated by a NO synthase inhibitor (Soejima et al., 1999). Thus, different chemical mediators may be involved in producing tissue damage developing at different stages of inhalation injury.

Concomitant with the acute airway exudative response, inhaled wood smoke induced a slight increase in Evans blue dye content in parenchymal tissues, an index of an increase in pulmonary microvascular permeability (Verbrugge et al., 1998). This pulmonary exudative response was also eliminated by pretreatment with CP-96,345 alone or in combination with dimethylthiourea, suggesting an origin similar to that of the airway exudative response. Both airway and pulmonary exudative responses seem to progress with time as judged by comparing responses measured at 5-min and 2-h post-airway exposure to wood smoke. It is known that tachykinins can increase the release of other inflammatory mediators (Barnes, 1996a). It has also been suggested that, as time progresses, smoke-induced inhalation injury may spread from the airways to the lung parenchyma via inflammatory mediators travelling through the bronchial circulation (Hales et al., 1989; Traber et al., 1992; Sakurai et al., 1998). The proinflammatory role of tachykinins in the pathogenesis of delayed smoke-induced lung edema is largely unknown and thus requires further investigation.

In conclusion, these results showed that both endogenous tachykinins and ·OH, but not arachidonate metabolites or NO, play an important role in producing smoke-induced acute airway plasma exudation and airway edema in guinea pigs. Additionally, the contribution of tachykinins to these two acute neurogenic airway responses is primarily mediated via the activation of tachykinin NK_1 receptors, is mediated in part by a ·OH mechanism, and is not associated with inactivation of airway neutral endopeptidase.

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