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Alleviation of wood smoke-induced lung injury by tachykinin receptor antagonist and hydroxyl radical scavenger in guinea pigs

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Abstract

We recently reported that wood smoke inhalation initially (within 5 min) causes airway injury and subsequently produces both airway and parenchymal injury after a delay (within 2 h). In this study, we investigated the mediator mechanisms of this delayed smoke-induced lung injury in 126 anesthetized and artificially ventilated guinea pigs who received challenges of either air or 40 tidal breaths of wood smoke. Two hours after inhalation, wood smoke produced various injurious responses, including increases in alveolar–capillary permeability, microvascular permeabilities, and histological injury scores, in airway and parenchymal tissues. Pre-treatment given before smoke challenge with CP-96,345 [a tachykinin NK_1 receptor antagonist; (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)-methyl)-1-aza bicyclo(2.2.2.)-octan-3-amine], dimethylthiourea (a hydroxyl radical scavenger), or a combination of these two drugs largely alleviated both the airway and parenchymal responses, whereas pre-treatment 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] or a combination of CP-96,344 and SR-48,965 (inactive enantiomers) failed to do so. Post-treatment given at 5 min after smoke challenge with CP-96,345 or dimethylthiourea significantly alleviated the parenchymal responses, while having no effect on the airway responses. Pre-treatment with dimethylthiourea prevented the smoke-induced reduction in airway neutral endopeptidase activity (an enzyme for tachykinin degradation). We concluded that (1) tachykinins and hydroxyl radical play important roles in producing smoke-induced delayed lung injury in guinea pigs, and both may be involved in the spread of injury from the airways to the pulmonary parenchyma, and (2) the contribution of tachykinins is mediated via the activation of tachykinin NK_1 receptors, and is associated with the hydroxyl radical-induced inactivation of airway neutral endopeptidase.

Keywords: Toxic smoke; Pulmonary edema; Tachykinin; Hydroxyl radical; Neutral endopeptidase

1. Introduction

Lung injury produced by toxic smoke has been recognized as a major cause of mortality associated with fire-related death (Crapo, 1981). Clinical and laboratory observations indicate that toxic smoke causes primary injury to the airways that advances with time to include the pulmonary parenchyma (Barrow et al., 1990; Moylan et al., 1972; Traber and Herndon, 1986). Several investigators (Kikuchi et al., 1996; Traber et al., 1992) have suggested that, during the initial phase following smoke inhalation, the injured airway tissues may release chemical mediators, which contribute to the subsequent parenchymal injury via bronchial circulation. While the developmental processes of toxic smoke-induced lung injury are relatively clear, the underlying mediator mechanisms are still largely unknown.

In a recent study (Lin and Kou, 2000), we demonstrated in guinea pigs that, within 5 min after wood smoke inhalation, prominent initial airway injury occurs, while the manifestations of pulmonary parenchymal injury are just about to become evident. Two hours after smoke inhalation, tissue injury progresses from the airways to the pulmonary parenchyma, resulting in delayed lung injury (Lin and Kou, 2000). Either a tachykinin receptor antagonist or a hydroxyl radical (·OH) scavenger largely attenuates the initial airway injury, suggesting the important role of tachykinins and ·OH in producing this response (Lin

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and Kou, 2000). However, the chemical mediators responsible for the lung (both airway and parenchyma) injury occurring in the delayed phase remain to be investigated.

Tachykinins are neuropeptides released from C-fiber sensory nerve endings (Barnes, 1996; Solway and Leff, 1991), whereas ·OH is an extremely reactive oxygen radical. Tachykinins and/or ·OH have been shown to evoke other airway responses to inhaled wood smoke, including sensory irritation (Kou et al., 1997; Lai and Kou, 1998), bronchoconstriction (Hsu et al., 1998a), and hyperreactivity (Hsu et al., 1998b, 2000). They are known as proinflammatory or inflammatory mediators that may be released to directly damage lung tissues under several pathophysiological conditions (Barnes, 1996; Solway and Leff, 1991; Ward et al., 1988). They may also increase the release of other chemical mediators, which are injurious to lung tissues (Barnes, 1996; Solway and Leff, 1991; Ward et al., 1988). Tachykinins produce their effects mainly via activation of tachykinin NK_1 and NK_2 receptors, and are rapidly degraded by neutral endopeptidase (Solway and Leff, 1991). This enzyme can be inhibited by oxygen radicals, leading to an amplification of pulmonary effects of tachykinins (Solway and Leff, 1991). It has been suggested that different chemical mediators may participate in different stages of smoke inhalation injury (Traber and Herndon, 1986). Therefore, the chemical mediators responsible for initial smoke-induced airway injury might not be the same as those producing delayed lung injury in our experimental model. Alternatively, after they have damaged airway tissues, tachykinins and ·OH may also produce delayed lung injury.

The present study was undertaken in anaesthetized guinea pigs to determine (1) whether tachykinins and ·OH are important in producing delayed smoke-induced lung injury in our guinea-pig experimental model and, if so, (2) whether they are the chemical mediators responsible for the spread of tissue injury from the airways to pulmonary parenchyma at the delayed phase, and (3) whether this lung injury is associated with ·OH-induced reduction in airway neutral endopeptidase activity.

2. Materials and methods

2.1. Animal preparation

Male Hartley guinea pigs (body weight, 322 ± 3 g; $n = 126$) were anesthetized with chloralose (100 mg kg^{-1}; i.p.; Sigma, St. Louis, MO, USA) and urethane (500 mg kg^{-1}, i.p.; Sigma). The carotid artery and jugular vein were cannulated to record arterial blood pressure to monitor the animal's condition and to administer pharmacological agents, respectively. Supplemental doses of chloralose (20 mg kg^{-1} h^{-1}) and urethane (100 mg kg^{-1} h^{-1}) were administered to maintain the abolition of pain reflexes induced by pinching the animal's hindpaw. The animals were ventilated with a respirator (model 683, Harvard Apparatus, South Natick, MA, USA) at a constant rate of 60 breaths min^{-1} via a tracheotomy. Tidal volume was adjusted in each animal (10 ml kg^{-1}) and was kept constant in each experiment. The animals were then paralyzed with pancuronium bromide (0.1 mg kg^{-1} h^{-1}, i.v.; Organon Teknika, Boxtel, Holland). 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 Guide 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, ROC.

2.2. Generation and delivery of wood smoke

The electric furnace and the methods for generating wood smoke are described in detail in our previous study (Kou and Lai, 1994). In brief, 100 g of dry wood dust (lauan wood) was thermally decomposed by the furnace (model 101, Nan Jou, Taipei, Taiwan, ROC) 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^{-1} 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 three-way stopcock. Wood smoke at a temperature of ~25 °C was delivered into the lungs by the respirator when the three-way stopcock was turned to communicate the respirator with the balloon. Before each smoke challenge, the lungs were hyperinflated ($4 \times$ tidal volume) to establish a constant volume history. For each smoke challenge, 40 tidal breaths of wood smoke were delivered. To avoid contamination, the expired smoke was drawn into a fume hood via a suction line.

2.3. Measurements of alveolar–capillary permeability

Alveolar–capillary permeability was assessed by measuring the Evans blue dye concentration in bronchoalveolar lavage fluid to serve as one of the indices of lung injury (Patterson et al., 1992; Verbrugge et al., 1998). Evans blue dye (25 mg kg^{-1}) was injected intravenously 1 min before the air or smoke challenge. Two hours later, the lungs were lavaged three times with a total amount of 15 ml warm saline. Each lavage was performed by introducing 5 ml of saline into the trachea cannula with three installations and withdrawals. The total volume of recovered lavage fluid was in the range of 12.6–13.2 ml. The lavage fluid was then centrifuged at $350 \times g$ for 20 min to remove cell and cell debris. The absorbance of supernatant fluid was determined (U-100, Hitachi) at 620 nm. The concentration of Evans blue dye in the fluid was calculated by interpolation on a standard curve of dye concentrations

in the range of 0.5–20.0 μg ml^{-1} and was expressed as μg ml^{-1}.

2.4. Measurements of tissue microvascular permeability

Tissue microvascular permeability was assessed by a method measuring the Evans blue dye extravasation described previously (Lin and Kou, 2000) to serve as one of the indices of lung injury (Verbrugge et al., 1998; Evans et al., 1988). Briefly, Evans blue dye (25 mg kg^{-1}) was injected intravenously 1 min before the air or smoke challenge. Two hours after airway challenge and after 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 (trachea, main bronchus, lobar bronchi, and several generations of segmental bronchi) was left. 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^{-1} and expressed as ng dye mg^{-1} of wet tissue.

2.5. 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. These sections were examined by a qualified pathologist in a blind fashion. The histological assessment was quantified by a scoring system that assigned a value from 0 (none or normal) to 3 (severe and/or diffuse) for each injurious variable of the airway and parenchymal tissues. The injurious variables assessed were epithelial shedding, submucosa edema, and an increase in cellularity in the airway tissues, and increases in the peribronchial cuff area, perivascular cuff area, thickness of the alveolar wall, and cellularity, and alveolar atelectasis and overdistension in the parenchymal tissues. Total injury scores for airway or lung parenchyma were obtained by adding the values assessed from these injurious variables. For each animal, two histological sections from the trachea and three sections from the pulmonary parenchyma were examined. For each section, structures at three different areas were randomly selected and their injury scores were averaged.

2.6. 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-naphthylaminie 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^{-1} of tissue protein h^{-1}.

2.7. Pharmacological interventions

A tachykinin NK_1 receptor antagonist [CP-96,345; (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)-methyl)-1-azabicyclo(2.2.2.)-octan-3-amine; 5 mg kg^{-1}; 5 mg ml^{-1}; Pfizer, Groton, CT, USA], an inactive enantiomer of CP-96,345 [CP-96,344, 5 mg kg^{-1}; 5 mg ml^{-1}; Pfizer], a tachykinin NK_2 receptor antagonist [SR-48,968; (*S*)-*N*-methyl-*N*(4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl)benzamide; 2 mg kg^{-1}; 1 mg ml^{-1}; Sanofi Research, Montpellier, France], and an enantiomer of SR-48,968 [SR-48,965, 2 mg kg^{-1}; 1 mg ml^{-1}; Sanofi Research] were each dissolved in isotonic saline and were injected as boluses into the vein. A ·OH scavenger (dimethylthiourea, 0.225 g kg^{-1} min^{-1}, 0.75 g ml^{-1}; Sigma) was dissolved in isotonic saline and was slowly infused into the vein for a period of 20 min. The doses of these drugs have been shown to effectively reduce various pulmonary pathophysiological responses to inhaled wood smoke in our previous studies (Lin and Kou, 2000; Hsu et al., 1998a,b, 2000).

2.8. Experimental procedures

A total of 126 animals were equally divided into 21 groups. In *Study 1*, nine groups of animals received a challenge of either air or 40 breaths of wood smoke, and their alveolar–capillary permeabilities were assessed 2 h after airway challenge. Among them, two groups were used to establish the air/saline vehicle and smoke/saline vehicle controls. Another five groups were used for the study of pre-treatment with either CP-96,345 alone, SR-48,968 alone, dimethylthiourea alone, CP-96,345 and dimethylthiourea in combination, or CP-96,344 and SR-48,965 in combination 10–15 min before smoke challenge. The remaining two groups were used for the study of post-treatment with either CP-96,345 or dimethylthiourea 5 min after smoke challenge. In *Study 2*, another nine groups had the same experimental procedures, and pharmacological pre- and post-treatments as those described in *Study 1*, except these animals were employed to measure microvascular permeabilities and total injury scores of the airways

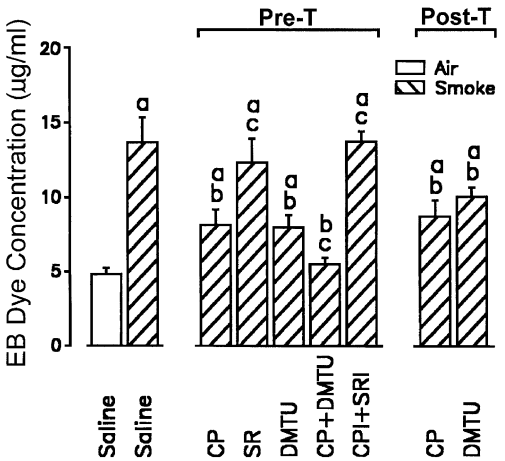

Fig. 1. Evans blue (EB) dye concentrations in bronchoalveolar lavage fluid sampled from animals exposed to air or wood smoke. Animals received pre-treatment (Pre-T) with saline vehicle, CP-96,345 alone (CP), SR-48,968 alone (SR), dimethylthiourea alone (DMTU), CP-96,345 and DMTU in combination (CP + DMTU), or CP-96,344 and SR-48,965 in combination (CPI + SRI), or received post-treatment (Post-T) with CP-96,345 alone or dimethylthiourea alone. [a]Significantly different from the air/saline control. [b]Significantly different from the smoke/saline control. [c]Significantly different from response to smoke in animals pre-treated with CP. Data in each group are means ± S.E. for six animals.

and parenchyma. This study was conducted to avoid the possibility that bronchoalveolar lavage performed in *Study 1* may complicate the smoke-induced lung injury. In *Study 3*, one group had pre-treatment with the saline vehicle and received sham air challenge, while the other two groups had a pre-treatment with saline vehicle or dimethylthiourea, and received 40 breaths of wood smoke. Two hours after the air or smoke exposure, the animals were killed with an overdose of anesthetics. Then the airway tissues, including the tracheal segment below the tip of the tracheal cannula and the main stem bronchi, were quickly removed, washed with isotonic saline, and stored at −70 °C for analysis of neutral endopeptidase activity.

2.9. Statistical analysis

Results of dye concentrations in lavage fluid, dye contents in tissues, and neutral endopeptidase activity were analyzed by a one-factor analysis of variance followed by Fisher's least significant difference procedure when appropriate. Data of total injury scores were evaluated by a Kruskal–Wallis nonparametric test followed by Mann–Whitney test when appropriate. $P < 0.05$ was considered significant. All data are presented as means ± S.E.

3. Results

3.1. Smoke-induced lung injury in control animals

Two hours after airway exposure, as compared to air controls, inhaled wood smoke significantly produced increases in both alveolar–capillary permeability and microvascular permeabilities of the airways and pulmonary parenchyma, as evidenced by increases in the dye concentration in lavage fluid (Fig. 1) and the dye contents in tissues (Fig. 2), respectively. Histological examinations revealed that smoke exposure produced substantial submucosa edema and an increase in cellularity in the airways, but caused no airway epithelial shedding (Fig. 3C). Additionally, wood smoke exposure produced large increases in the peribronchial cuff area, perivascular cuff area, thickness of the alveolar wall, and cellularity, and caused notable alveolar atelectasis and alveolar overdistension in the parenchymal tissues (Fig. 3D). In contrast, these injurious signs were seldom seen in the airways (Fig. 3A) and parenchyma (Fig. 3B) of animals exposed to air. Overall, the total injury scores of the airways and parenchyma assessed in the smoke-exposed animals were significantly greater than those in the air-exposed animals (Fig. 4).

3.2. Effects of pre-treatments on smoke-induced lung injury

In animals pre-treated with either CP-96,345 alone, dimethylthiourea alone, or CP-96,345 and dimethylth-

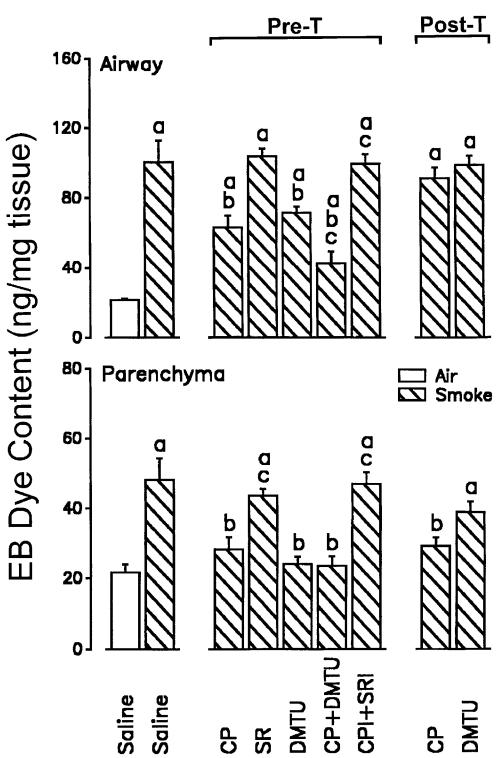

Fig. 2. Evans blue (EB) dye contents of airway and pulmonary parenchymal tissues excised from animals exposed to air or wood smoke. Animals received pre-treatment (Pre-T) with saline vehicle, CP-96,345 alone (CP), SR-48,968 alone (SR), dimethylthiourea alone (DMTU), CP-96,345 and DMTU in combination (CP + DMTU), or CP-96,344 and SR-48,965 in combination (CPI + SRI), or received post-treatment (Post-T) with CP-96,345 alone or dimethylthiourea alone. [a]Significantly different from the air/saline control; [b]Significantly different from the smoke/saline control; [c]Significantly different from response to smoke in animals pre-treated with CP. Data in each group are means ± S.E. for six animals.

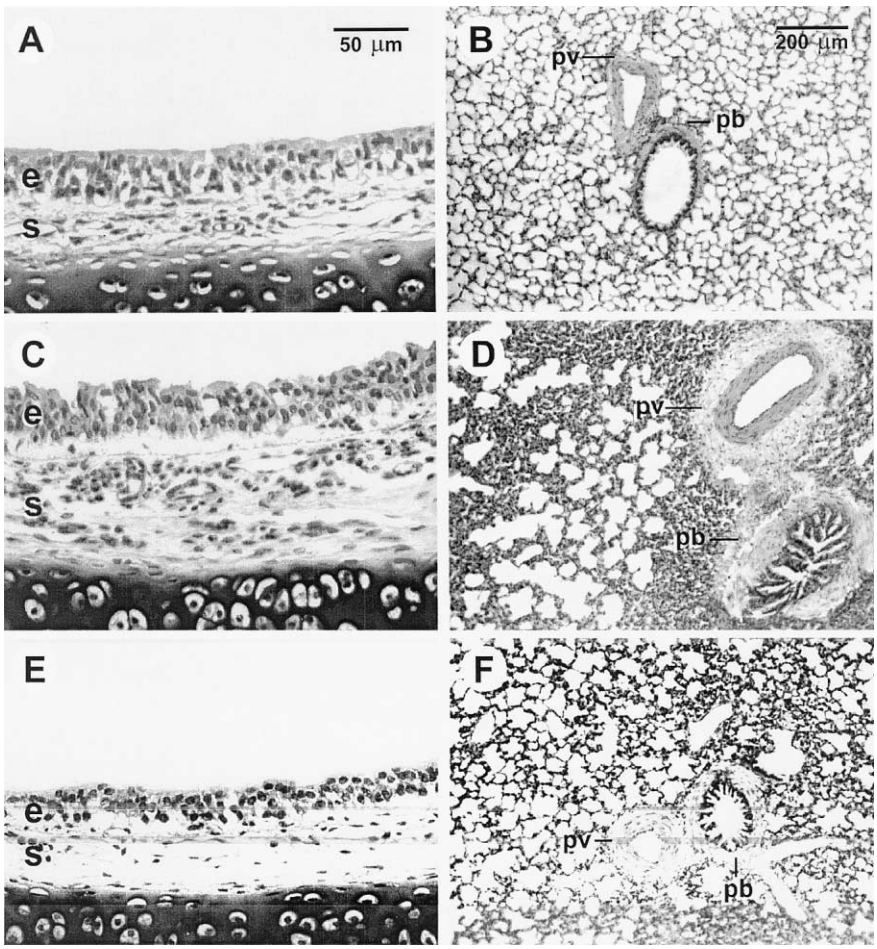

Fig. 3. Light micrographs of trachea (A, C, and E) and pulmonary parenchyma (B, D, and F) excised from animals exposed to air (A and B), to wood smoke (C and D), and to wood smoke with pre-treatment with CP-96,345 and dimethylthiourea in combination (E and F). e, epithelium; s, submucosal space; pb, peribronchial cuff; pv, perivascular cuff. Magnification: 400 × in A, C, and E; 100 × in B, D, and F.

iourea in combination, the smoke-induced increases in dye concentration in lavage fluid (Fig. 1) and dye contents in airway and parenchymal tissues (Fig. 2) were significantly smaller than the smoke/saline controls. Additionally, pre-treatment with CP-96,345 and dimethylthiourea in combination resulted in a further attenuation of increases in dye concentration in lavage fluid (Fig. 1) and dye content in airway tissues (Fig. 2), as compared to the group pre-treated with CP-96,345 alone. Histological examinations revealed that these three pre-treatments alleviated each of the pathological signs of airway and parenchymal injury (Fig. 3E and F). The alleviations were more effective in tissue edematous responses and cellularity, but less effective to alveolar overdistension and atelectasis. Overall, the total injury scores of the airway and parenchymal tissues assessed in the smoke-exposed animals with these pre-treatments were significantly smaller than those assessed in the smoke-exposed control animals (Fig. 4). In sharp contrast, in animals pre-treated with either SR-48,968 alone or CP-96,344 and SR-48,965 in combination, the above mentioned injurious responses did not significantly differ from the smoke/saline controls (Figs. 1, 2 and 4).

3.3. Effects of post-treatments on smoke-induced lung injury

In animals post-treated with either CP-96,345 or dimethylthiourea, the smoke-induced increases in the dye concentration in lavage fluid (Fig. 1), the dye content in parenchymal tissues (Fig. 2), and the total injury score of the lung parenchyma (Fig. 4) were significantly smaller than the smoke/saline controls. Conversely, the increase in the dye content in airway tissues (Fig. 2) and the total injury score of the airways (Fig. 4) assessed in animals with these post-treatments did not significantly differ from the smoke/saline controls. Histological examinations revealed that these two post-treatments effectively alleviated each of the pathological signs of parenchymal injury, yet they failed to alter the pathological signs of airway injury.

3.4. Effects of wood smoke on airway neutral endopeptidase activity

In animals pre-treated with saline, neutral endopeptidase activity measured in airway tissues excised 2 h after

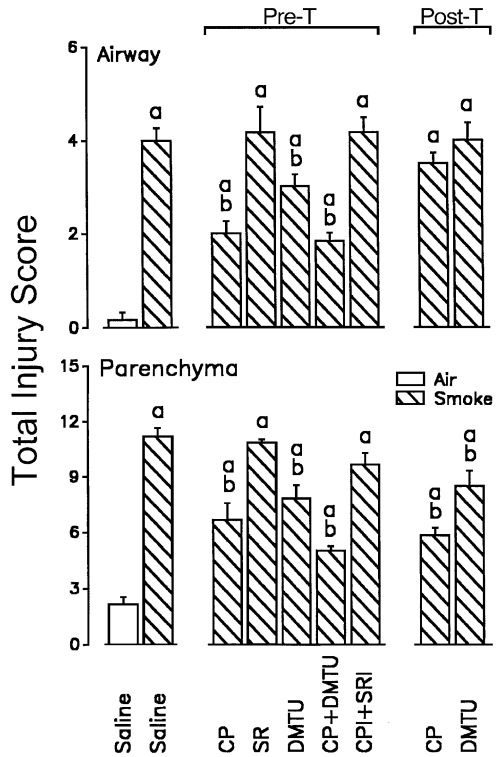

Fig. 4. Total injury scores of airway and pulmonary parenchymal tissues excised from animals exposed to air or wood smoke. Animals received pre-treatment (Pre-T) with saline vehicle, CP-96,345 alone (CP), SR-48,968 alone (SR), dimethylthiourea alone (DMTU), CP-96,345 and DMTU in combination (CP + DMTU), or CP-96,344 and SR-48,965 in combination (CPI + SRI), or received post-treatment (Post-T) with CP-96,345 alone or dimethylthiourea alone. [a]Significantly different from the air/saline control. [b]Significantly different from the smoke/saline control; [c]Significantly different from response to smoke in animals pre-treated with CP. Data in each group are means ± S.E. for six animals.

wood smoke challenge (31.5 ± 3.7 nmol mg^{-1} protein h^{-1}; $n = 6$; $P < 0.05$) was significantly lower than that in tissues excised at the same time after the sham air challenge (53.6 ± 5.9 nmol mg^{-1} protein h^{-1}; $n = 6$). This smoke-induced reduction in neutral endopeptidase activity was totally prevented in animals pre-treated with dimethylthiourea (60.4 ± 6.0 nmol mg^{-1} protein h^{-1}; $n = 6$; $P > 0.05$).

4. Discussion

In this study, we demonstrate that, within 2 h after smoke inhalation, delivery of 40 tidal breaths of wood smoke induces a prominent delayed lung injury in anesthetized guinea pigs. This delayed lung injury was evidenced by increases in alveolar–capillary permeability, and in microvascular permeabilities and total injury scores of both the airway and pulmonary parenchymal tissues. This delayed lung injury contrasts to our previous findings (Lin and Kou, 2000) that, within 5 min after smoke inhalation, delivery of the same amount of wood smoke induces an initial airway injury, while having little effect on the pulmonary parenchyma. Evidently, from the initial to the delayed phase, the airway injury persists and parenchymal injuries are encompassed as time progresses. These smoke effects are similar to those reported from clinical and animal studies (Barrow et al., 1990; Moylan et al., 1972; Traber and Herndon, 1986).

We found that pre-treatment with either CP-96,345 or dimethylthiourea, nearly abolishes or largely reduces the injurious responses of both the airways and pulmonary parenchyma occurring in the delayed phase, whereas pre-treatment with either SR-48,968 or a combination of CP-96,344 and SR-48,965 fails to do so. These results suggest that tachykinins and ·OH play an important role in delayed smoke-induced lung injury, and that the contribution of tachykinins is primarily mediated through the activation of tachykinin NK_1 receptors. However, we have previously demonstrated that these two chemical mediators also play a similar role in producing airway injury occurring in the initial phase (Lin and Kou, 2000). Therefore, after the airways have been initially damaged by tachykinins and ·OH, either these two chemical mediators themselves, or some other chemical mediators released from the injured airway tissues, may be involved in the development of the delayed parenchymal injury. In either case, our pre-treatment interventions first prevented the initial airway injury and then the ensuing delayed lung injury. Thus, while the importance of tachykinins and ·OH can be recognized from the results of pre-treatment, we are unable to differentiate which possibility is likely. We then studied these injurious responses in animals post-treated with either CP-96,345 or dimethylthiourea 5 min after smoke inhalation, a time when the initial airway injury had occurred, but the manifestations of the delayed lung injury were just about to become evident (Lin and Kou, 2000). We found that these two post-treatments largely reduced the parenchymal responses, while having no effects on alleviating the airway responses developed at the delayed phase. These results suggest that tachykinins and ·OH are the key mediators responsible for the spread of tissue injury from the airways to the pulmonary parenchyma. The inability of post-treatments to alleviate airway injury certainly reflects the intractableness of this injury when airways have been damaged by toxic smoke. However, the effectiveness of post-treatments to prevent parenchymal injury in the delayed phase is relevant to a promising therapy for patients with early signs of smoke inhalation injury.

Alleviation of the pathological signs of lung injury achieved by CP-96,345 and dimethylthiourea was more effective toward tissue edematous responses and the increase in cellularity, but less so toward alveolar overdistension and atelectasis. Both tachykinins and ·OH are known to have direct injurious effects on the microvasculature (Evans et al., 1988; Rubanyi, 1988). It is known that tachykinin-associated edematous responses are predominantly mediated through the activation of tachykinin NK_1 receptors (Barnes, 1996; Solway and Leff, 1991), a view-

point that is consistent with the present observations. Hence, reducing leakage of the microvasculature caused by inhaled wood smoke would alleviate its resultant edematous and inflammatory responses (Chung et al., 1990). Although oxygen radicals are known to inhibit the surfactant function (Gilliard et al., 1994), it is conceivable that factors other than tachykinins and ·OH are involved in developing smoke-induced alveolar atelectasis and overdistension.

The tachykinins that produce the observed lung injury presumably originate from an increase in their release from lung sensory C-fiber nerve endings when wood smoke stimulates these pulmonary receptors (Kou et al., 1995; Lai and Kou, 1998). On the other hand, ·OH may have both exogenous and endogenous origins. Wood smoke contains high concentrations of free radicals and radical precursors which are formed during combustion (Pryor, 1992). Furthermore, ·OH may be formed and released endogenously by certain inflammatory cells when they are activated by smoke inhalation (Traber and Herndon, 1986). Despite the fact that their contributions are clear, the exact mechanisms by which tachykinins and ·OH participate in the development of delayed smoke-induced lung injury remain unclear, but are speculated to be multifactorial. For example, either reduction of the bronchial circulation in a sheep model (Abdi et al., 1991; Kramer et al., 1989; Sakurai et al., 1998) or ligation of the bronchial artery in a dog model (Efimova et al., 2000; Hales et al., 1989) attenuates edema formation in the lung occurring after lung injury with toxic smoke inhalation, suggesting the important contribution of bronchial circulation to the response. Additionally, toxic smoke inhalation to the right lung results in damage to the air-insufflated left lung in a sheep model, indicating hematogenous mediation of the response (Kikuchi et al., 1996). These observations support the notion that toxic smoke may initially damage the airways and increase the release of chemical mediators from injured tissues, which contribute to subsequent parenchymal injury via bronchial circulation (Kikuchi et al., 1996; Traber et al., 1992). Although not confirmed, it is plausible that the same mechanism may work in our guinea pig model. If so, after they initially damage the airways, tachykinins and ·OH might enter the bronchial circulation with subsequent emptying into the pulmonary microvasculature to produce parenchymal injury, as suggested by other investigators (Traber et al., 1992).

As compared to pre-treatment with CP-96,345 alone, pre-treatment with CP-96,345 and dimethylthiourea in combination further attenuates some of the injurious responses, while having no additive effect on the others. Therefore, tachykinins and ·OH might act independently or dependently in different lung tissues to produce the observed injurious responses. The possibility that these two mediators act dependently is supported by the fact that oxygen radicals may inhibit neutral endopeptidase and exaggerate pulmonary effects of tachykinins (Solway and Leff, 1991). Indeed, we found that the activity of airway neutral endopeptidase in guinea pigs was reduced 2 h after wood smoke inhalation. This reduction in neutral endopeptidase activity was largely prevented by pre-treatment with dimethylthiourea, suggesting that ·OH is the major chemical factor responsible for this reduction. This notion is consistent with our previous findings (Hsu et al., 2000). We previously found that the airway neutral endopeptidase activity was not significantly affected in airway tissues excised 5 min after delivery of 40 breaths of wood smoke (Lin and Kou, 2000). Therefore, it would be assumed that the time after smoke insult could be an important factor for the inactivation of airway neutral endopeptidase by inhaled wood smoke. Another possibility 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 lung C-fiber sensory nerve endings by inhaled wood smoke (Kou et al., 1997; Lai and Kou, 1998). Therefore, scavenging ·OH by dimethylthiourea would reduce the amount of the release of C fiber-containing tachykinins available for producing the observed lung injury. Furthermore, 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). Hence, blocking tachykinin receptors by their antagonist may possibly reduce the contributions from both tachykinins and ·OH. Oxygen radicals have been largely implicated in the development of toxic smoke-induced lung injury (Kimura et al., 1988; Lalonde et al., 1994; Nguyen et al., 1995; Youn et al., 1992). The present findings further suggest the possibility that oxygen radicals might work together with tachykinins to produce smoke-induced lung injury. Apart from tachykinins and ·OH, the role of other chemical mediators in producing the smoke-induced lung injury has been investigated in several animal models. For example, leukotriene (Hales et al., 1995; Janssens et al., 1994) and interleukin-8 (Laffon et al., 1999) have been reported to play a significant role. The possible functional interactions between other chemical mediators and tachykinins or ·OH require further investigation.

In summary, our results from the study of pharmacological pre-treatment show that both endogenous tachykinins and ·OH play important roles in producing smoke-induced airway and parenchymal injury in guinea pigs. Additionally, the contribution of tachykinins to these responses is mediated via the activation of tachykinin NK_1 receptors and is associated with the ·OH-induced inactivation of airway neutral endopeptidase.

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