Tomato juice prevents senescence-accelerated mouse P1 strain from developing emphysema induced by chronic exposure to tobacco smoke

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Submitted 29 December 2004; accepted in final form 24 September 2005

EMPHYSEMA AND INFLAMMATION in small airways due to chronic tobacco smoke are cardinal pathological findings in chronic obstructive pulmonary disease (COPD), a disease state characterized by airflow limitation. The prevailing theory for the pathogenesis of emphysema is a proteinase/antiproteinase imbalance theory that was deduced from a clinical observation of patients with α1-antitrypsin deficiency who are at increased risk of early onset emphysema (14). Tobacco smoke exposure induces airway and parenchymal inflammation by recruiting and activating inflammatory cells to release proteinases, particularly elastase from neutrophils and various metalloproteinases from alveolar macrophages, which will overwhelm the antiproteinase defense in the epithelial lining fluid and lung tissues. However, the mechanisms leading to tobacco smoke-induced emphysema are not so simple, and recent advances revealed that not only various cells and mediators but also different biological processes are involved in the pathogenesis of emphysema, including the imbalance between oxidant stress and antioxidant defenses in the lungs (24, 43) or between cellular apoptosis and regeneration (3, 42, 43).

To elucidate the pathogenesis of COPD, there have been several animal models for emphysema reported in the literature (33), including a classic model instilling proteinases via airways, a genetically engineered mouse to overexpress collagenase (12), interferon-γ (44), inducible targeting of IL-13 (45), transgenic disruption of klotho (36), and so on. Several groups reported the successful development of smoke-induced emphysema in mice (10, 16, 25, 35), although they took a long period of smoke exposure (generally 6 or 7 mo) to generate emphysema. Among these animal models for emphysema, klotho mice are unique since it shows various phenotypes of aging such as arteriosclerosis, osteoporosis, skin atrophy, and ectopic calcifications (22). However, klotho mice show emphysema early at 4 wk of age without smoke exposure (36).

The senescence-accelerated mouse (SAM) strains, established by Takeda et al. (37), are naturally occurring animal models for accelerated aging after normal development and maturation and have been widely used as models for investigating aging processes including senile cataract (39), amyloidosis (38), osteoporosis (27), senile lung (23, 40), and age-related impairment in memory and learning (29). Among several substrains of senescence-prone mouse strain (SAMP), both SAMP1 and SAMP2 strains were reported to manifest the functional and morphological aspects of senile lung with increasing age: age-related air space enlargement without significant parenchymal destruction and hyperinflation with the increase of lung compliance (23, 40). Because COPD in humans usually occurs in elder populations with a long period of smoking history, we postulated that aging of the lung could be an important factor and should be incorporated into an experimental animal model of smoke-induced emphysema.

Carotenoids, common dietary constituents and naturally contained in most fruits (orange or yellow) and vegetables (green leaves), are known to exert antioxidant activities and prevent free radical-induced cellular damage (4). A large body of research has investigated the potential role of antioxidant...
nutrients in prevention of chronic diseases and aging processes in humans (28). Lycopene is a major carotenoid in tomatoes (20) and is considered to be the most efficient biological carotenoid in quenching singlet oxygen (13). Since tobacco smoke contains more than 10$^{15}$ oxidant molecules/puff (7), it is conceivable that dietary carotenoid intake may influence the development of tobacco smoke-induced emphysema.

In this study, we investigated the lung pathology of SAMP1 mice exposed to tobacco smoke for 8 wk and found that this short period of exposure was sufficient to reproducibly generate emphysema. We also conducted a preventive experiment utilizing this model system to show whether ad libitum ingestion of tomato juice containing lycopene abundantly prevents SAMP1 mice from the development of smoke-induced emphysema.

**MATERIALS AND METHODS**

**Animals.** Several pairs of SAMP1/Ka and SAMR1/Ta (senescence-resistant control) strains were provided from the Council for SAM Research, bred in the Animal Research Facility of Juntendo University School of Medicine, and used throughout this study. All procedures were approved by the Animal Ethics Committee of Juntendo University. All mice were maintained in a limited-access barrier and housed in a humidity- (55 ± 10%) and temperature- (24 ± 2°C) controlled room under a 12-h light and 12-h dark cycle. All mice were provided with a standard commercial chow (CRF-1; Oriental Kobo, Tokyo, Japan) and were allowed free access to food and water. When lycopene was administered to mice, tap water was replaced by tomato juice (5 mg lycopene, 52.6 mg vitamin A, 0.68 g protein, 0 g lipid, 0.68 g fiber, 110 mg sodium, 279 mg potassium, 6.8 mg calcium, and 20 kcal in 100 g of tomato juice) that was diluted with an equal volume of water to reduce its density. Diluted tomato juice was freshly prepared every other day and replaced the old one. There was no significant difference in the amount of ingestion of the commercial chow observed whether water or diluted tomato juice was provided.

**Chronic inhalation of tobacco smoke.** Inhalation of tobacco smoke was performed utilizing unfiltered research cigarette 1R3F (Tobacco Health Research Institute, Kentucky Univ., Lexington, KY) and the Tobacco Smoke Inhalation Experiment System for Small Animals (model SIS-CS; Shibata Scientific Technology, Tokyo, Japan). Model SIS-CS consisted of both a tobacco smoke generator (model SG-200) and an inhalation chamber. The smoke generator was controlled by a laptop computer and automatically generated tobacco smoke by setting a volume of syringe pump (10–50 cm$^3$/puff) and a number of puffs/min (1–12 puffs). The tobacco smoke generated is delivered to the inhalation chamber, to which the mouse body holders are set (maximum 12 body holders can be set at a time), and mice inhale tobacco smoke through their noses. Tobacco smoke can be diluted to a desired concentration at the blinder, where tobacco smoke and compressed air were combined, before delivery to the inhalation chamber. At 12 wk of age, mice were subjected to the experiment of chronic inhalation of tobacco smoke. We used the following experimental settings in this study: 15 ml of stroke volume and 12 puffs/min to generate tobacco smoke, 1.5% tobacco smoke diluted by compressed air. The mass concentration of total particulate matter (TPM) in 1.5% tobacco smoke was determined by gravimetric analysis of filter samples taken during exposure periods and was 23.9 mg of TPM/m$^3$.

First, mice were trained to be set into a body holder for 30 min/day without smoking for 5 days, followed by exposure to 1.5% tobacco smoke for 15 min/day for 5 days. After this induction period, mice continued to inhale 1.5% tobacco for 30 min/day, 5 days/wk, and for 8 wk. As a control for the inhalation of tobacco smoke, mice were subjected to the same experimental procedure with the same conditions described above, but air was delivered instead of tobacco smoke.

**Bronchoalveolar lavage and morphometric evaluation of the lungs.** Mice were weighed after anesthesia with intraperitoneal injection of pentobarbital sodium in saline (10 mg/100 g body wt). After the main pulmonary artery was cannulated with a 20-gauge Teflon catheter, mice were killed by exsanguination through the left atrium with perfusion of PBS through the main pulmonary artery. The lungs were removed and lavaged with 0.5 ml of PBS through an intratracheal cannula four times. The bronchoalveolar lavage fluid (BALF) from each mouse lung was pooled and measured for recovery rate. Total cell counts in each bronchoalveolar lavage were calculated with a hemocytometer, and cell populations were determined after staining with a modified Wright stain (Diff-Quick fixative, Midori Jyushi).

After bronchoalveolar lavage was performed, the lungs were inflated and fixed by intratracheal instillation of 20% buffered formalin (pH 7.4) at a constant pressure of 25 cmH$_2$O for 24 h. After routine processing of successive dehydration, the lungs were embedded in paraffin and sectioned in a frontal plane at the depth of hilum, followed by a further cut of each block into two equally sized blocks in the frontal plane. Histological section was prepared at 4-μm thickness from three planes of four blocks and stained with hematoxylin and eosin.

The change of air space size was assessed by the determination of the mean linear intercepts (MLI) according to the method described by Thurlbeck (41). Ten randomly selected fields in each section at $\times 100$ magnification were used for the calculation of MLI. The destructive index (DI) was calculated to evaluate the destruction of the alveolar wall according to the method described by Saetta et al. (32). Ten randomly selected fields in each section at $\times 50$ magnification were utilized to measure DI. A DI >10% was considered to have a significant destruction of lung parenchyma (40).

**Determination of lycopene concentration in serum and the lungs.** The concentration of lycopene in the serum and lung tissues was determined by high-performance liquid chromatography as described previously (30).

**Evaluation of apoptosis by immunohistochemistry for anti-single strand DNA and anti-activated caspase-3 antibodies.** Apoptosis of lung cells was examined by immunohistochemistry using a rabbit polyclonal antibody against single-stranded DNA (ssDNA) (Dako Cytomation). The lung tissue slides were washed twice with cold PBS, incubated in normal goat serum for 30 min, and followed by incubation for 1 h with anti-ssDNA antibody at a 1:100 dilution. Antibody binding was detected using a biotinylated anti-rabbit IgG antibody (Dako Cytomation), a horseradish peroxidase-conjugated streptavidin (Dako Cytomation), and 3,3’-diaminobenzidine tetrachloride as the chromogen. The percentage of apoptotic cells (ratio of positively immunostained nuclei to total count of the nuclei present in the field at $\times 200$ magnification) was determined in three different areas from three planes of the lung per mouse: the parenchymal area (>500 nuclei of alveolar cells counted per mouse), bronchiolar cells adjacent to alveolar dust (>300 nuclei), and bronchial cells in the central airways (>300 nuclei).

Activated caspase-3 was detected using a rabbit polyclonal antibody against cleaved caspase-3 antibody (Cell Signalling) following the manufacturer’s instruction. Briefly, the lung tissue slides were washed twice with cold PBS and immersed into boiling citrate buffer for 30 min. The slides were cooled for 30 min, incubated in methanol containing 3% hydrogen peroxide for 10 min, and followed by incubation with 5% goat serum for 1 h at room temperature. After being washed, the slides were incubated with anti-cleaved caspase-3 antibody (1:200 dilution) overnight at 4°C. Antibody binding was detected by the same manner described above.

**Determination of VEGF in lung tissues.** The lung tissues were homogenized in PBS, and supernatant was obtained by ultracentrifugation at 11,100 g for 10 min at 4°C. VEGF concentration in the supernatant was determined using a commercially available ELISA kit (Quantikine Mouse VEGF kit; R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.
Determination of total glutathione in lung tissues. The lungs were homogenized in 5% 5-sulfosalicylic acid, and the supernatant was obtained by centrifugation at 12,000 rpm for 10 min at 4°C. Glutathione concentration in the supernatant was measured using Total Glutathione Quantification kit (Dojindo Molecular Technologies, Gaithersburg, MD) according to the manufacturer’s instructions.

Protein assay. Protein concentration in the lung supernatant was determined using BCA Protein Assay kit (Pierce Biotechnology) according to the manufacturer’s instructions.

Statistical analysis. Statistical analysis was performed using two-way ANOVA and Bonferroni multiple comparison post hoc pairwise comparison when significant differences were found. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effect of chronic tobacco exposure in both SAMR1 and SAMP1 strains. Body weights of mice did not change significantly in both SAMR1 and SAMP1 strains before and after exposure to tobacco smoke for 8 wk. In addition, tobacco smoke had no influence on body weight gain in both strains during the experiment (data not shown). After an 8-wk exposure to air or tobacco smoke, total cell counts and cell population in BALF did not differ significantly in both SAMR1 and SAMP1 strains between air-exposed and tobacco smoke-exposed groups (Table 1). Bronchoalveolar lavage cells were composed mainly of macrophages and neutrophils that were barely detected even after tobacco smoke exposure for 8 wk.

We observed the difference in baseline characteristics of the lungs between SAMP1 and SAMR1. After 8 wk of exposure to air as a control for tobacco smoke (5 mo of age), there was a significant increase of MLI (7.7%; SAMP1 vs. SAMR1, 68.7 ± 0.7 vs. 63.8 ± 1.2 μm; $P < 0.05$; Fig. 1). In addition, we detected the greater DI in SAMP1 than SAMR1, but still less than a cut-off value.

After an 8-wk exposure to tobacco smoke, the MLI was significantly increased in the SAMP1 strain (9.5%) compared with air-exposed SAMP1 ($P \leq 0.05$; Fig. 1A). In contrast, there was no significant change of MLI detected in the control SAMR1 strain exposed to tobacco smoke compared with air-exposed SAMR1. The DI was increased >10%, a cut-off value indicating the occurrence of significant alveolar destruction (40), in the tobacco-exposed SAMP1 strain, whereas there was no relevant increase of DI recognized in the SAMR1 strain (Fig. 1B). Together, these results indicate that emphysema developed in SAMP1, but not in SAMR1, after tobacco smoke exposure under our experimental condition.

We measured glutathione as a major antioxidant in the lung (31) as well as VEGF, a growth factor being important to maintain the structural integrity of lung parenchyma (18), in the lung homogenate of SAMP1 and SAMR1 (Fig. 2). The baseline level of lung glutathione (air-exposed mice) showed a tendency to be higher in SAMP1 than SAMR1 ($P = 0.0546$), but its lung content significantly increased in response to tobacco smoke exposure in SAMR1, whereas SAMP1 did not show a significant response (Fig. 2A). On the other hand, the baseline level of VEGF was significantly lower in the lungs of SAMP1 compared with those of SAMR1. Moreover, VEGF decreased in SAMP1 after tobacco smoke exposure, whereas it did not in SAMR1 (Fig. 2B). These results suggest that SAMP1 and SAMR1 strains have different baseline characteristics of the lungs as well as response to chronic tobacco smoke exposure.

Effect of tomato juice on tobacco smoke-induced emphysema in SAMP1 strain. We examined the effect of concomitant administration of tomato juice on the development of tobacco smoke-induced emphysema. The SAMP1 strain was given tap water or tomato juice during the period of smoke exposure experiment. Smoke exposure itself did not have any effect on body weight or its increase during the period but resulted in a slight but significant decreased body weight and its gain when tomato juice was concomitantly given together with smoke exposure (Table 2). Histopathological and morphometric analysis clearly demonstrated emphysematous change in tobacco smoke-exposed SAMP1 ingesting tap water but not in SAMP1 ingesting tomato juice (Fig. 3). The MLI was reproducibly increased in tobacco-exposed SAMP1 compared with air-exposed SAMP1 ($P < 0.05$) when tomato juice was not administered, but the increase of MLI was completely prevented by concomitant ingestion of tomato juice ($P < 0.05$; Fig. 3, A and C). In contrast, the increase of DI was significantly prevented by tomato juice, but the DI did not return to the control level (Fig. 3B).

Lycopene was detected in both serum and lung tissues when mice ingested tomato juice for 1 and 4 wk instead of tap water (Table 3). No significant difference was observed in lycopene concentration of both serum and lung tissue at 1 or 4 wk before air- and smoke-exposed SAMP1 except that serum lycopene at 1 wk was significantly decreased in smoke-exposed SAMP1 compared with air-exposed SAMP1. The longer the period the mice ingested tomato juice, the more lycopene appeared to accumulate in the lungs and reach a similar level as with serum. There was no lycopene detected in serum and lungs when tomato juice was not given to SAMP1.

VEGF in the lung homogenate was reproducibly decreased after tobacco smoke exposure, but tomato juice prevented the decrease of VEGF associated with smoke exposure and even showed a tendency to upregulate VEGF in smoke-exposed SAMP1 (Fig. 4). However, the value of VEGF content in the lung was much higher than the previous experiment presented (Fig. 2B). We cannot explain the exact reasons for this fluctuation of data, but it may due to our inconsistent technique to exsanguinate blood from the lungs or some individual variations among SAMP1 mice.

Table 1. Cell populations in bronchoalveolar lavage fluid after tobacco smoke exposure

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Total Cell Number ($\times 10^4$ cells/ml)</th>
<th>%Macrophages</th>
<th>%Neutrophils</th>
<th>%Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMR1 (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>12.1 ± 2.9</td>
<td>97.8 ± 1.1</td>
<td>0.4 ± 0.4</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Smoke</td>
<td>15.9 ± 4.2</td>
<td>99.3 ± 0.5</td>
<td>0</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>SAMP1 (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>14.5 ± 3.6</td>
<td>96.6 ± 1.5</td>
<td>0</td>
<td>3.4 ± 1.5</td>
</tr>
<tr>
<td>Smoke</td>
<td>18.3 ± 4.6</td>
<td>97.8 ± 1.6</td>
<td>0.2 ± 0.2</td>
<td>2.0 ± 1.4</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Senescence-prone strain (SAMP1) and control (SAMR1) mice were exposed to air or tobacco smoke for 8 wk, and bronchoalveolar lavage was performed (see MATERIALS and METHODS). There was no statistically significant difference in total cell number and cell population between air-exposed and smoke-exposed mice in both strains: in SAMP1, total cell number, air vs. smoke exposed, $P = 0.4465$; in SAMR1, $P = 0.5306$. Similarly, no difference was noted in total cell number and cell population between air-exposed SAMP1 and SAMR1 or between smoke-exposed SAMR1 and SAMP1; total cell number, air-exposed SAMR1 vs. SAMP1, $P = 0.6513$; smoke-exposed SAMP1 vs. SAMR1, $P = 0.6886$. 

AJP-Lung Cell Mol Physiol • VOL 290 • FEBRUARY 2006 • www.ajplung.org
Apoptosis of lung cells. Apoptosis of lung cells was analyzed to investigate the mechanisms of tobacco smoke-induced emphysema in SAMP1. Immunohistochemical examination using anti-ssDNA antibody demonstrated that apoptosis was enhanced by tobacco smoke but significantly reduced by the administration of tomato juice (Fig. 5, A and B). Apoptosis was widely detected in bronchial and bronchiolar epithelial cells and alveolar septal cells. Quantification of immunoreactive nuclei revealed that chronic tobacco smoke exposure enhanced apoptosis in all areas compared with those in air-exposed SAMP1. However, the administration of tomato juice ameliorated the tobacco smoke-induced increase of apoptosis equally in all areas (P < 0.01; Fig. 5, A and B). Tobacco smoke-induced increase of apoptosis was further confirmed by the immunohistochemical analysis of caspase-3: activated caspase-3 was demonstrated in lung specimen of smoke-exposed SAMP1 without tomato juice (Fig. 5C) but not in those with tomato juice (data not shown).

DISCUSSION

We have shown that the SAMP1 strain is susceptible to tobacco smoke exposure for 8 wk and reproducibly developed emphysema, whereas concomitant administration of
lycopene, given as tomato juice, completely prevented SAMP1 mice from developing smoke-induced emphysema.

SAMP strains are unique mice showing an accelerated senescence after normal development and maturation (37) and have been widely used as a model for investigating the aging process (27, 29, 38, 39). The SAMP1 strain was reported as a murine model for senile hyperinflation lung since several morphometric parameters, including lung volume, MLI, total alveolar duct air volume, and total alveolar air volume, increase with age in an accelerated manner, and the significant differences in these parameters between SAMP1 and the control SAMR1 strain became evident at 6–17 mo of age (23). In this context, our experimental model, tobacco smoke-induced emphysema in SAMP1 strain, is a unique animal model since this strain intrinsically has an accelerated aging process of the lungs. Because COPD in humans usually occurs in the elderly population with a history of a long period of smoking, we considered that aging of the lung could be an important factor and should be incorporated into an experimental animal model of COPD. We postulated the age-related characteristics of the lungs in SAMP1 enabled us to generate smoke-induced emphysema in a short period, whereas other groups required a long period of smoke exposure (6–7 mo) to generate smoke-induced emphysema (10, 16, 25, 35). In other words, our study suggested that aging is an important factor for smoke-induced emphysema to develop. However, it did not exclude the possibility that the different conditions for smoke generation and exposure we employed might enable us to induce emphysema in a relatively short period.

The SAMP1 mice we utilized in this study showed features of accelerated aging, as another study reported, but there may be slight substrain differences. Kurozumi et al. (23) measured the MLI at 1, 2, 6, 10, and 17 mo of age in SAMP1 and reported that air space enlargement was evident after 6 mo of age. They did not measure the DI in SAMP1, although another substrain of a model for a senile lung, SAMP2, was reported to show no difference in DI compared with SAMR1 (40). In our study, we detected air space enlargement in SAMP1 at 5 mo of age, at the end of an 8-wk exposure started at 3 mo of age. In addition, we detected greater DI in SAMP1 than SAMR1 but still less than a cut-off value. The exact mechanisms behind the accelerated aging in SAM remain undetermined. We found a different baseline content of glutathione and VEGF in the lung tissues as well as different responses to chronic tobacco smoke exposure between SAMP1 and SAMR1. In the SAMR1 control strain, glutathione in the lung tissues significantly increased after tobacco smoke exposure for 8 wk. Oxidative stress generated by tobacco smoke may initially deplete glutathione, followed by a rebound increase of glutathione in chronic smoke exposure as an adaptive response to oxidative stress, which occurs as a result of upregulation of γ-glutamylcysteine synthetase (31). Accordingly, a significant increase of lung glutathione content in SAMR1 control strain appears to indicate that our experimental system of chronic tobacco smoke exposure appropriately delivered oxidant burdens contained in tobacco smoke to mouse lungs and altered oxidant-antioxidant balance in the lungs. In contrast with SAMR1, a trend was evident for increased baseline glutathione without further increase in response to chronic tobacco smoke exposure in SAMP1 lung tissues. Because oxidative stress is widely believed to be involved in the aging process (18, 19), SAMP1 may be in a state such that γ-glutamylcysteine synthetase has been already upregulated by intrinsic oxidative stress and cannot respond anymore to smoke exposure. Similarly, VEGF, an important growth factor for lung parenchyma (18), demonstrated a difference in both a baseline level and its response to chronic tobacco smoke exposure. SAMP1 had a lower baseline level of VEGF than SAMR1 and decreasing response of VEGF to smoke exposure, whereas SAMR1 maintained the same VEGF level as baseline after smoke exposure. Together, these differences may be important factors contributing to both the phenotypical differences of the lungs between SAMP1 and SAMR1 and the earlier development of smoke-induced emphysema in SAMP1.

The pathogenesis of tobacco smoke-induced emphysema in humans is now under intensive investigation. In animal models of smoke-induced emphysema, TNF-α-mediated inflammatory responses, the influx of inflammatory cells including neutrophils and macrophages that in turn release various proteinases such as neutrophil elastase and metalloproteinases, have been reported to contribute to pulmonary parenchymal destruction (8–10, 16, 35). In our model utilizing the SAMP1 strain, however, this does not appear to be the case. We found that the cell population in BALF, composed mainly of macrophages and neutrophils, was barely detected even after tobacco smoke exposure for 8 wk (Table 1). Furthermore, we could not detect an increase of TNF-α in BALF of smoke-exposed SAMP1 after an 8-wk exposure (data not shown). Accordingly, we postulated another mechanism by which smoke-induced emphysema could be generated in the SAMP1 strain and focused on the oxidant-antioxidant imbalance (24, 43). In addition, oxidative stress is widely believed to be involved in the aging process (18, 19). In this context, lycopene, a potent antioxidant abundantly contained in tomato juice (13, 20), was utilized to test the hypothesis that oxidant-antioxidant imbalance could be important in our model rather than inflammatory cell influx with increased burden of proteinases.

We have successfully confirmed that tomato juice comitantly given with chronic tobacco smoke exposure completely protected SAMP1 mice from smoke-induced
emphysema. Because lycopene was evidently detected in both serum and lung tissues when tomato juice was ingested by mice, we expect that lycopene modulates the oxidant-antioxidant balance perturbed by our experimental condition of chronic tobacco smoke exposure. Neither chronic smoke exposure nor ingestion of tomato juice itself had a significant effect on body weight increase during the 8-wk experimental period in SAMP1, but body weight slightly, but significantly, decreased in a smoke-exposed group with tomato juice ingestion. This decrease of body weight would not have any association with the prevention of emphysema since body weight loss associated with calorie restriction resulted in air space enlargement in mice rather than influencing the decrease of alveolar size (26). In this context, we postulated that an oxidant-antioxidant imbalance was one of operating mechanisms on smoke-induced emphysema in the SAMP1 strain. However, we have to be careful to interpret whether our experimental system truly generated oxidative stress in SAMP1 since we did not measure any other markers of oxidative stress except for glutathione. Possibilities remain that the increase of glutathione in the lung merely reflects chronic smoking rather than oxidative stress.

Fig. 3. Morphometric and histological findings of the lung tissues of SAMP1 after exposure to air or tobacco smoke with or without administration of tomato juice. A: MLI. Data were expressed as means ± SE (n = 6 in each group). Open and filled bars indicate samples without or with administration of tomato juice, respectively. B: DI. Data are presented in the same manner as A (n = 6 in each strain). C: representative histological views (n = 6 in each group) were presented (hematoxylin and eosin stain, original magnification, ×20). Note that tobacco exposure for 8 wk generated air space enlargement (a), but the administration of tomato juice prevented mice from developing emphysema (b).
and that there may not exist a parallel change of glutathione content in response to tobacco smoke exposure between the two compartments, BAL and lung tissues, as those reported in inflammatory cell populations and many cytokines (11). Moreover, another group reported that glutathione content and its response to smoke exposure may be dependent on the mouse strain (6), as further illustrated between SAMP1 and SAMR1 in the present study.

We observed increased apoptosis associated with chronic smoke exposure in airway epithelial and alveolar septal cells. It is widely accepted that free radicals and reactive oxygen species delivered by cigarette smoke directly damage cells and induce apoptosis (1, 2, 7). Apoptotic nuclei positively immunostained by anti-ssDNA antibody increased in all three areas we examined in smoke-exposed SAMP1 compared with the air-exposed group and decreased significantly by concomitant ingestion of tomato juice. Apoptosis itself would not evoke inflammatory cell responses, but recent studies indicate that direct instillation of activated caspase-3 generated emphysema (3) and the blockade of vascular endothelial growth factor signaling by VEGF-receptor antagonist, resulting in apoptosis of alveolar septal cells (21). These reports clearly provide evidence that apoptosis of lung cells, especially alveolar epithelial and endothelial cells, causes emphysema without apparent inflammation. Several lines of evidence indicated the interaction between oxidative stress and apoptosis where a vicious cycle may be established: cells undergoing apoptosis show increased oxidative stress that further contributes to apoptosis (17, 43). In interactions between oxidative stress and apoptosis regarding emphysema, VEGF, a growth factor abundantly expressed in the lung and exerting a survival signal for endothelial cells, is considered a key molecule (43). In accordance with observations that cigarette smoke extract downregulates VEGF expression by epithelial cells (43), VEGF in the lung tissues of smoke-exposed SAMP1 significantly decreased. Accordingly, the reduction of VEGF concentration could have some role in the apoptosis of alveolar septal cells in our smoke-induced emphysema model. However, other mechanisms are likely to be involved in apoptosis of lung cells since tomato juice completely prevented VEGF in the SAMP1 lung tissues from decreasing by tobacco smoke exposure and even appeared to upregulate VEGF in the lungs, but apoptosis of lung cells did not return to the control level. Because mice were given tomato juice instead of a pure lycopene preparation, we cannot exclude the possibility that other ingredients contained in tomato juice affected the results, including the increase of VEGF in smoke-exposed SAMP1 lung tissues.

There have been several animal models for COPD reported in the literature (34) and studies (6, 15) reporting the strain-dependent susceptibility for the development of emphysema induced by tobacco smoke exposure. But, to our knowledge, the mice in which the klotho gene was disrupted (36) is the only model intrinsically possessing the aging factor in their biological background. However, this mouse model would not be appropriate as an analogy of tobacco smoke-induced emphysema in elderly humans since klotho mice develop emphysema without smoke exposure. Hence, it would not be utilized for interventional experiments for innovating preventive or therapeutic agents as an analogy of human diseases. We have shown that smoke-induced emphysema in the SAMP1 strain was completely prevented with the concomitant administration of lycopene given as tomato juice. Our model appears to have a potential application for various in vivo experiments not only in understanding the pathophysiology of smoke-induced emphysema but also in performing interventional projects.

ACKNOWLEDGMENTS

We thank Dr. Toshio Kumasaka, Department of Pathology, Juntendo University School of Medicine, for pathological evaluation and technical assistance and Dr. Noriyoshi Sueyoshi, Division of Biomedical Imaging Research, Juntendo University School of Medicine, for technical assistance.

GRANTS

This study was supported by Grant-in-Aid for Scientific Research Nos. 13470130 (Y. Fukuchi) and 15390259 (Y. Fukuchi), the High Technology Research Center Grant from the Ministry of Education, Culture, Sports, Finance, and Technology, Japan.

Table 3. Concentration of lycopene in serum and lungs of SAMP1 mice

<table>
<thead>
<tr>
<th>Period</th>
<th>Serum (ng/ml)</th>
<th>P</th>
<th>Lung (ng/g tissue)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Air</td>
<td>13.25±3.24</td>
<td>&lt;0.05</td>
<td>1.11±0.16</td>
</tr>
<tr>
<td></td>
<td>Smoke</td>
<td>4.49±1.83</td>
<td></td>
<td>1.07±1.07</td>
</tr>
<tr>
<td>4</td>
<td>Air</td>
<td>9.45±2.30</td>
<td>=0.3349</td>
<td>8.61±2.09</td>
</tr>
<tr>
<td></td>
<td>Smoke</td>
<td>5.79±2.05</td>
<td></td>
<td>6.84±1.24</td>
</tr>
</tbody>
</table>

Data are means ± SE (n = 4 in each group). Tomato juice was given instead of tap water for 1 or 4 wk with exposure to air or tobacco smoke. Lycopene content in lung tissue significantly increased at 4 wk compared with that at 1 wk in both air-exposed (P < 0.01) and smoked-exposed (P < 0.01) SAMP1. However, there was no significant difference noted in serum lycopene concentration of air-exposed or smoke-exposed SAMP1 between 1 and 4 wk.

Fig. 4. The content of VEGF in SAMP1 lung tissues after tobacco smoke exposure with or without tomato juice ingestion. Lung tissues were homogenized after exposure to air or tobacco smoke for 8 wk with (filled bars) or without (open bars) administration of tomato juice. Data are presented as means ± SE (n = 6 in each group).
Fig. 5. Immunohistochemical detection of apoptosis in the lung tissues of SAMP1 after exposure to air or tobacco smoke with or without administration of tomato juice. A: immunoreactive nuclei for anti-single-stranded DNA (ssDNA) antibody were counted in 3 areas and expressed as positive ratio (%) of total nuclei counted. AC, alveolar septal cells; PB, bronchiolar cells adjacent to alveolar duct; CB, bronchial cells in the central airway. Data were expressed as means ± SE (n = 6 in each group). *P < 0.01, significantly decreased compared with smoke-exposed SAMP1 without tomato juice. †P < 0.05, significantly decreased compared with air-exposed SAMP1 without tomato juice. B: representative results of immunohistochemistry for ssDNA (original magnification, ×20). Insets are magnified views of alveolar region (×100). Note that positive immunostaining (nuclear brown staining) for DNA strand breaks was revealed in airway epithelium and alveolar wall cells of tobacco-exposed lung compared with control and that concomitant administration of tomato juice markedly reduced positive immunostaining of lung cells in tobacco-exposed lung. C: representative results of immunohistochemistry for active caspase-3 in tobacco-exposed tomato juice (−) SAMP1 (original magnification, ×200).
REFERENCES