Cigarette smoking and smoking cessation restores laryngeal mucus secretory homeostasis in rats

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Abstract

Introduction: The mechanisms underlying the effects of cigarette smoke and smoking cessation on respiratory secretion, especially in the larynx, remain unclear.
Objectives: The aims of this study were to determine the effects of cigarette smoke and smoking cessation on laryngeal mucus secretion and inflammation, and to investigate the effects of glucocorticoid administration.
Methods: We administered cigarette smoke solution (CSS) to eight-week-old male Sprague Dawley rats for four weeks, then examined laryngeal mucus secretion and inflammatory cytokine expression on days 1, 28 and 90 after smoking cessation. We also investigated the effects of the glucocorticoid triamcinolone acetonide when administered on day 1 after smoking cessation.
Results: Exposure to CSS resulted in an increase in laryngeal mucus secretion that was further excacerbated following smoking cessation. This change coincided with an increase in the expression of mRNA for the inflammatory cytokines tumor necrosis factor and interleukin-6, as well as mRNA for MUC5AC, which is involved in mucin production. Both mucus secretion and inflammatory cytokine expression had decreased to control levels by 90 days after smoking cessation. Triamcinolone acetonide suppressed CSS-induced laryngeal mucus hypersecretion and pro-inflammatory cytokine production.
Conclusion: Cigarette smoke-associated inflammation may contribute to the exacerbated laryngeal mucus hypersecretion that occurs following smoking cessation. The inflammatory response represents a promising target for the treatment of cigarette smoke-associated mucus hypersecretion.

Objectives

A long history of cigarette smoking significantly increases the risk of various mucus hypersecretion diseases in the respiratory organs, such as chronic laryngitis and chronic obstructive pulmonary disease (COPD). In addition to causing mucus hypersecretion, cigarette smoke impairs mucus excretion by destroying the cilia that move mucus from the lung to the throat. As a result, mucus accumulates in the respiratory tracts of smokers, irritating the sensitive tissue therein and leading to 'smoker's cough'.

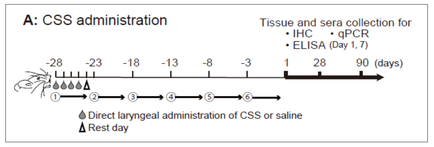
Previous studies of the effects of cigarette smoking on mucus production have mainly focused on sections of the lower respiratory tract such as the trachea, bronchial tubes, and alveolus. However, the upper respiratory tract, especially the larynx, is also an important target of cigarette smoking in terms of the discomfort associated with excessive larynx or the relationship between discomfort and phlegm in the throat after smoking. However, to our knowledge, no studies have examined the effects of cigarette smoking on laryngeal mucus secretion. In addition, the changes in inflammatory responses and mucus production that occur after smoking cessation remain largely unknown, though the association between cigarette smoking and inflammatory markers and mediators is well known.

Glucocorticoids are an important class of anti-inflammatory and immunosuppressive drugs that negatively modulate inflammatory gene expression and that are typically used for the treatment of chronic inflammatory diseases. The glucocorticoid triamcinolone acetonide (TA) has long-lasting effects due to its synovial atrophying properties and slow absorption from the site of administration. Clinically, TA treatment is effective against a variety of chronic inflammatory diseases such as chronic rhinosinusitis and nasal polyposis, COPD, and arthritis. Because of the association between cigarette smoking and airway inflammation, anti-inflammatory therapies may improve the laryngeal mucus secretion and chronic cough that are suffered by patients following smoking cessation.

In this study, we examined the effects of smoking, short-term (four-week) smoking cessation (SC), and long-term (three-month) SC on laryngeal secretion and inflammatory responses using a rat model of smoking. We also investigated the effects of steroid administration on laryngeal secretion and inflammatory responses.

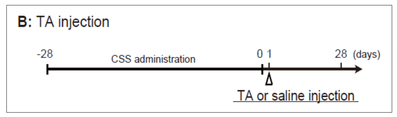
Methods

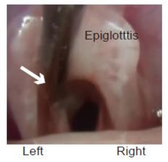
I. Rat model of smoking



- Sprague Dawley rats（8W, male)
- Cigarette smoke solution (CSS; 1 μl/ g/ time) .

II. Drug injection procedure

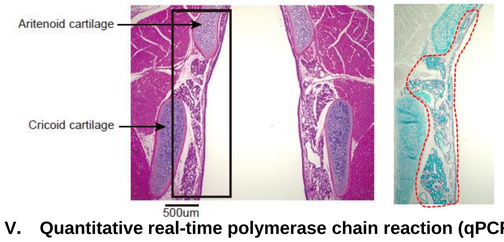




- Triamcinolone acetonide (TA; 0.2 μg/g/ time)
  - TA, one of glucocorticoids, have a long-lasting effect, and is effective for chronic inflammatory diseases.

IV. Histological analyses

- H&E staining: Evaluation of whole tissue structure
- Alcian blue staining: Observation of the mucus and goblet cells.
- To compare the amount of mucus, the cross-sectional area of Alcian blue-positive mucus and goblet cells over the perichondrium of thyroid and cricoid cartilages (boxed area) were measured using image analysis software (Micro Analyzer Ver. 1.1 Nippon Poladigital, Japan).

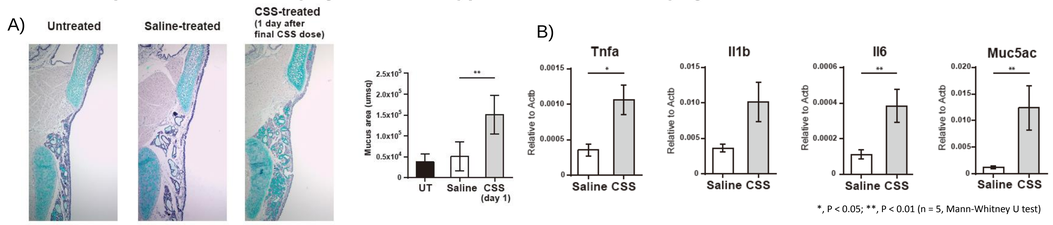


V. Quantitative real-time polymerase chain reaction (qPCR)

Pro-inflammatory cytokines; ***TNF-α***, ***IL-1β***, ***IL6***,
Mucin; ***MUC5AC***

Results

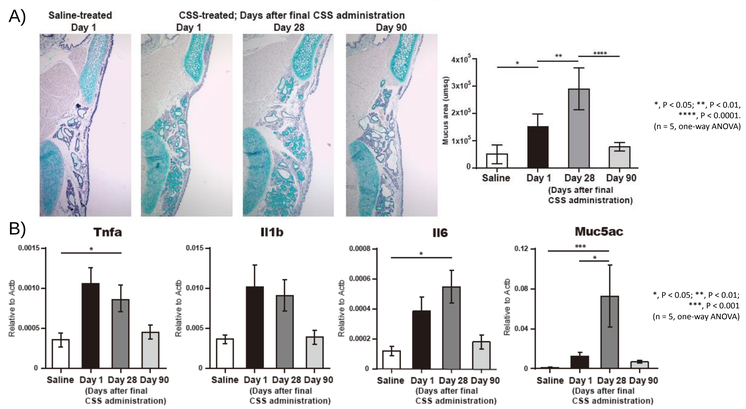
I. CSS exposure induces laryngeal mucus hypersecretion and laryngeal inflammation



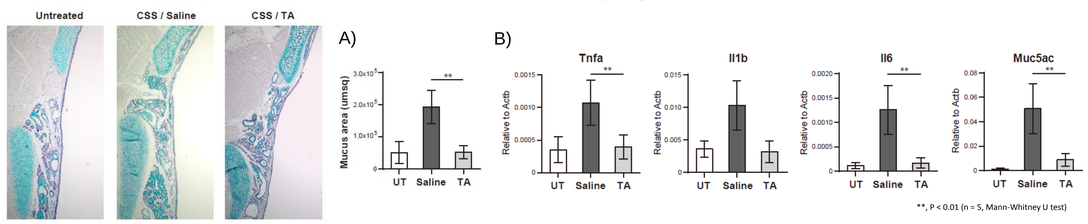
A) The Alcian blue-positive mucus secreting area was significantly increased in CSS-treated rats.
B) Expression of *Tnfa*, *Il6* and *Muc5ac* mRNA was higher in the infralaryngeal tissues of CSS-treated rats than saline-treated rats.

II. Laryngeal mucus hypersecretion is transiently exacerbated after smoking cessation (SC)

A) The mucus secreting area in the laryngeal mucosa increased significantly between day 1 and day 28 after cessation of CSS administration, but returned to saline-treated levels by day 90.
B) Expression of Muc5ac mRNA increased considerably between day 1 and day 28 after cessation of CSS exposure consistent with changes in laryngeal histology.
- SC resulted in a transient exacerbation in laryngeal mucus secretion and inflammation, but these conditions were reversible and resolved within 90 days of the final exposure to CSS.



III. Triamcinolone acetonide (TA) suppresses CSS-induced laryngeal mucus hypersecretion, pro-inflammatory cytokine production and Muc5ac mRNA upregulation



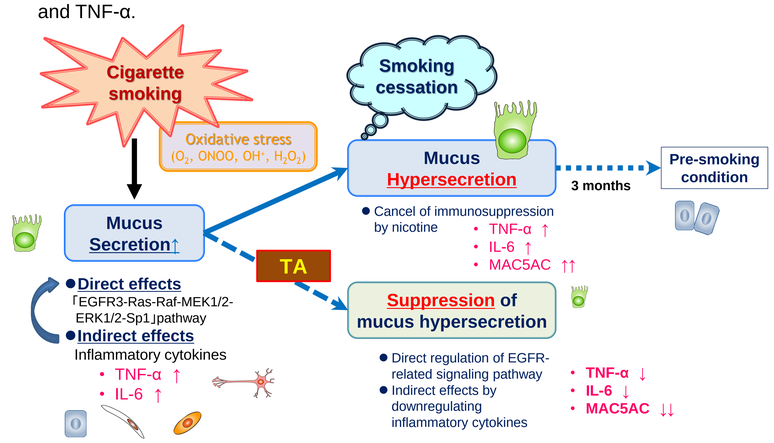
A) Laryngeal mucus secretion was significantly lower in TA-treated rats than in saline-treated control rats.
B) *Tnfa*, *Il6* and *Muc5ac* mRNA expression was lower in TA-treated rats compared to the saline-treated control rats, though the decrease of Il1b mRNA expression was not statistically significant.

Discussions

- CSS-induced mucin hypersecretion and Muc5ac overexpression are considered to be due to
  i. direct effects on transcriptional regulation of epidermal growth factor receptor(EGFR)-related signaling pathway (EGFR-Ras-Raf-MEK1/2-ERK1/2-Sp1)
  ii. indirect effects through inflammatory reactions

- Nicotine, a major constituent of cigarette smoke, has immunosuppressive effects, and the decrease of nicotine concentration due to SC possibly reverses nicotine-induced suppression of the inflammation in the larynx.

- TA inhibits all stages of the inflammatory response, and reduces airway mucus production and the proliferation of epithelial goblet cells. In addition, TA induces repression of NF-kβ activation caused by smoking, and directly regulate EGFR.
  - TA might be effective to suppress laryngeal mucus hypersecretion by directly regulating EGFR-related signaling pathway and by downregulating inflammatory cytokines; IL-6, and TNF-α.



Conclusion

- We have demonstrated that CSS induces laryngeal hypersecretion, besides short-term SC causes further hypersecretion and upregulation of pro-inflammatory cytokines and *Muc5ac* mRNA.
- The inflammatory responses and mucus hypersecretion that occur during smoke exposure and after SC represent promising targets for the treatment of cigarette smoke-associated prolonged mucus hypersecretion.
- TA may reduce expression of pro-inflammatory cytokines in the larynx and might be useful for the treatment of CS-induced mucus hypersecretion, which may cause prolonged cough after SC.

Refersnces

➢R. Ueha, S. Mukherjee, et al. "Viral disruption of olfactory progenitors is exacerbated in allergic mice." Int Immunopharmacol 2014 **22**(1): 242-247.
➢R. Ueha, S. Ueha, et al. "Damage to olfactory progenitor cells is involved in cigarette smoke-induced olfactory dysfunction in mice." Am J Pathol 2016 **186**(3): 579-586.
➢R. Ueha, S. Ueha, et al. "Cigarette Smoke Delays Regeneration of the Olfactory Epithelium in Mice." Neurotoxitcity Research 2016 **30**(2): 213-224..

Funding

This work was supported by a Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (number 24791749) and by the Smoking Research Foundation (Tokyo, Japan).

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