Functional residual capacity and airway resistance in rats of COPD model induced by systemic hyaluronidase

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***Objective*: Chronic obstructive pulmonary disease (COPD) is characterized by obstructive bronchiolitis and parenchymal destruction. In animal models, air space enlargement induced by intratracheal elastase is augmented by prior depletion of lung hyaluronan by hyaluronisase. Recently our colleagues reported that intravenous hyaluronisaein in the absence of elastase produced emphysema-like alveolar dilation [1]. In this study we measured functional residual capacity (FRC) and airway resistance (R_{aw}) in the rats with hyaluronidase-induced experimental COPD.**
***Materials and Methods*: Hyaruonidase (20 mg/kg) was administered from the caudal vein of 19 male Wistar rats (*COPD rats*). Two weeks after the injection, FRC and R_{aw} were measured with bodyplethysmogarph.**
***Results*: Thickness or inflammatory cell infiltrations were not apparent in the bronchus of the *COPD rat* while alveolar distension was obvious. The mean FRC of the *COPD rats* (6.22 ± 1.00 ml, mean ± SD) was significantly larger than that of *Control rats* (5.48 ± 0.85 ml). There was no statistical significance between the mean R_{aw} of the COPD rats (0.28 ± 0.08 cmH_2O/ml/s) and that of the control rats (0.28 ± 0.13 cmH_2O/ml/s).**
***Conclusion*: Systemic administration of hyaluronidase produced pulmonary overinflation but did not bronchial constriction. We speculate that hyaluonidase-induced COPD simulates panlobular emphysema.**

Key words: COPD, animal model, hyaluronidase, pulmonary function

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. Current definition of COPD is a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases. The chronic airflow limitation characteristic of COPD is caused by a mixture of obstructive bronchiolitis and parenchymal destruction [2]. Distension and rupture of alveolar walls in COPD is believed to result from the destruction of elastic fibers in the lung interstitium. Elastases from neutrophils and macrophages are now thought to play an important role in the pathogenesis of COPD [2]. Hyaluronidase is normally present in the lung and helps regulate the turnover of hyaluronan, a large extracellular meshwork of proteoglycans [3]. In experimental COPD, air space enlargement induced by intratracheal elastase is augmented by prior depletion of lung hyaluronan by intratracheally administered hyaluronisase in rats [4]. Furthermore, our colleagues have reported that intravenous hyaluronisae, in the absence of elastase or hyperoxia, produceed emphysema-like alveolar dilation [1]. However, in their model pulmonary overinflation and bronchial obstruction, which are characteristic of COPD, have not been estimated. Therefore, aim of the present study was to establish hyaluronidase-induced COPD model by quantitative measurement of the two representative parameters of COPD, i.e., functional residual capacity (FRC) and airway resistance (R_{aw}).

MATERIALS AND METHOD

The experimental subjects were 38 male Wistar rats that were 10 weeks of age. Nineteen of the rats were administered hyaruonidase (20 mg/kg in normal saline, Sigma, H4272) from the caudal vein (*COPD rats*). The remaining 19 rats were administered normal saline as controls (*Control rats*). Both group rats lived after 2 weeks of injection and then FRC and R_{aw} were measured. Details of the measurements were described elsewhere [5]. In brief, after pentobarbital i.p. injection (0.05 mg/g), tracheal intubation with a plastic tube (2.1 mm OD) was performed. The rats were then placed in the supine position in the bodyplethysmograph, inner milieu of which was maintained at body temperature and atmospheric pressure, i.e., BTPS. The FRC was calculated from changes in the airway pressure against the changes in the pressure in the bodyplethysmograph during airway closure at FRC [6].

The airway resistance was obtained from Ohm's low (circuit resistance = driving pressure/flow) when the rat is lying in the bodyplethysmograph and is breathing without airway closure. Thus the R_{aw} was calculated from the pressure swings in the bodyplethysmograph which represented alveolar pressure, against the flow during spontaneous breathing.

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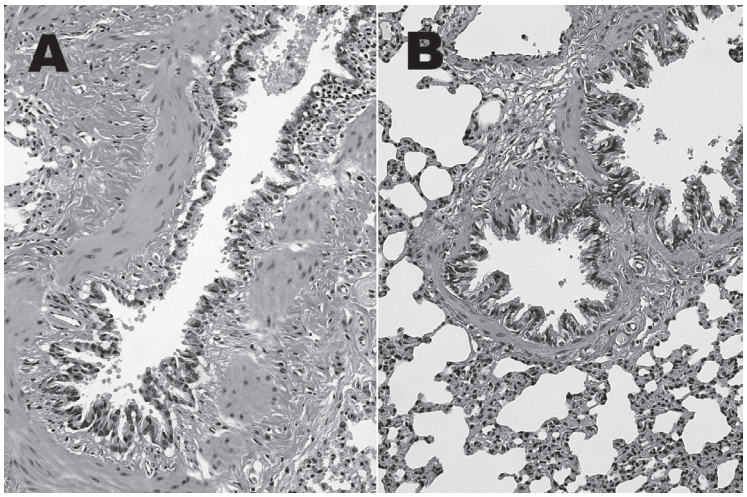

Fig. 1 The bronchial wall of a control group rat (A) and that of a hyaluronidase-treated rat (B). There was no apparent thickness or inflammatory cell infiltration in the bronchus of the COPD rat. H & E stained. original magnification × 150.

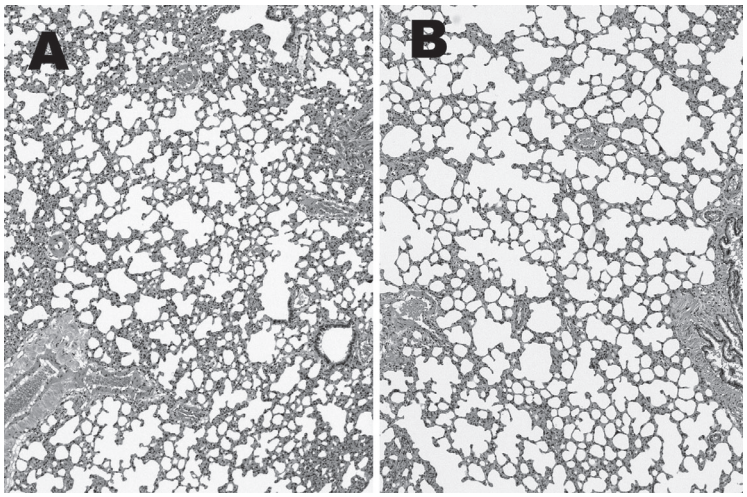

Fig. 2 The lung of a control group rat (A) and that of a hyaluronidase-treated rat (B). Alveolar distension was apparent in hyaluronidase-treated rat. H & E stain. original magnification × 60.

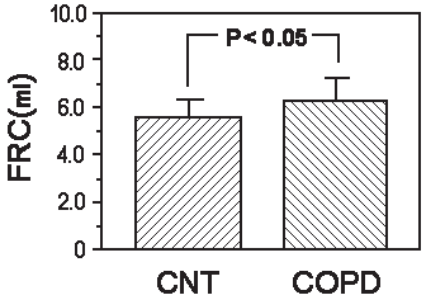

Fig. 3 Mean FRC of the control group rat (CNT) and that of the hyaluronidase-treated rat (COPD). Bars, SD.

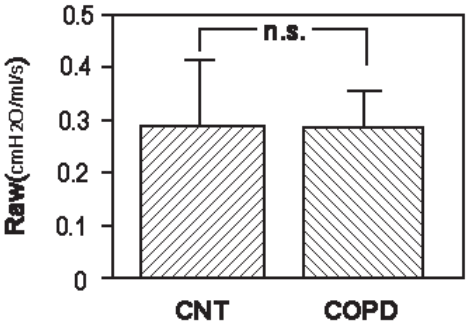

Fig. 4 Mean R_{aw} of the control group rat (CNT) and that of the hyaluronidase-treated rat (COPD). Bars, SD.

group rats were killed with overdose of pentobarbital i.p. for the preparation of lung section. Rat lung tissues were processed for routine paraffin embedding, and serial sections (5 μm) were stained with hematoxylin and eosin (H & E). Because of small sample numbers statistical analysis was not done on these specimens.

The results were expressed as mean ± SD. If the difference of the measured values between the two group rats was $P < 0.05$ by paired *t* test, the difference was regarded as significant.

RESULTS

The mean bodyweight of *Control Rats* at the measurements was 281 ± 19 g and that of *COPD Rats* was 286 ± 29 g. There was no significant difference between them.

Figure 1 shows H & E stain of the bronchus of a *COPD Rat* comparing with that of a *Control Rat*. There was no apparent thickness or inflammatory cell infiltrations in the bronchus of the *COPD rat*.

Figure 2 shows H &E stain of the lung of a *COPD Rat* comparing with that of a *Control Rat*. As has been reported by Shioya *et al*. [1] alveolar distension was apparent in *COPD rat*.

Figure 3 shows FRCs of the two group rats. The mean FRC of the *Control rats* was 5.48 ± 0.85 ml and that of *COPD rats* was 6.22 ± 1.00 ml. Mean FRC of the *COPD rats* was significantly larger than that of *Control rats*.

Figure 4 shows R_{aw}s of the two group rats. The mean R_{aw} of the control rats was 0.28 ± 0.13 cmH_2O/ml/s and that of COPD rats was 0.28 ± 0.08 cmH_2O/ml/s. There was no statistical significance between them.

DISCUSSION

In the present functional study on an experimental model of COPD induced by intravenous hyaluroni-

dase, we found significant increase in lung volume but no apparent increase in airway resistance.

Hyaluronan mitigates the action of elastases such as porcine pancreatic elastase, as well as human neutrophil elastase and human macrophage metaprotease. This action has been demonstrated in vivo in models of pulmonary emphysema [3]. Air space enlargement induced by intratracheal elastase is augmented by prior depletion of lung hyaluronan by intratracheally administered hyaluronidase [4, 7]. Whether depletion of hyaluronan itself induces experimental emphysema has not been proven. However, Shioya *et al* found in their preliminary study that intravenous hyaluronidase, in the absence of elastase or hyperoxia, produced enlargement of alveolar spaces [1]. They analyzed free water in the lung of these animal models using time domain reflectometry but functional study was yet to be investigated. The present study demonstrated that alveolar enlargement produced by intravenous hyaluronidase was associated with pulmonary overinflation which is a characteristic feature of COPD.

In addition to a role in protecting against alveolar destruction, hyaluonan has also been found to block bronchial obstruction induced by aerosol administration of pancreatic elastase in sheep [8]. Because *in vitro* experiments have demonstrated that hyaluronan can inactivate tissue kallikrein, the protective effect of hyaluronan against elastase-induced bronchoconstriction is thought to be mediated through inactivation of tissue kallikrein. Contradictory to these notions, airway resistance did not increase in hyaluronidase-induced experimental COPD rats in the present study. This finding is not surprising because in clinical practice COPD caused by alpha-1 antytripsin deficiency does not cause panlobular emphysema but centrilobular emphysema [2]. Presumably systemic imbalance between protease and antiprotease shifted to destruction of alveolar tissue rather than bronchoconstriction. This speculation could be clarified by future study comparing lung tissue of experimental COPD induced by intravenously hyaluronidase with that by intratracheal hyaluronidase.

In conclusion, this functional study revealed that intravenous hyaluronidase produced pulmonary overinflation that confirms the previous morphological study but not bronchial constriction. The COPD model induced by systemic hyaluronidase may simulate panlobular emphysema.

REFERENCES

1) Shioya S, Tsuji C, Hashimoto M, *et al*. Measurement of tissue free water in the experimental emphysema. J Jpn Respir Soc 41(supp): 196, 2003.
2) Global Initiative for Chronic Obstructive Lung Disease. National Institute of Health. National Heart, Lung and Blood Institute updated 2005. http://goldcopd.com/
3) Turino GM, Cantor JO. Hyaluronan in respiratory injury and repair. Am J Respir Crit Care Med 167: 1169-1175, 2003.
4) Murakami H, Yoshiba M, Aritomi T, *et al*., Effects of hyaluronidase on porcine pancreatic elastase-induced lung injury. J Jpn Respir Soc 36: 557-584, 1998.
5) Tajiri S, Kondo T. Direct measurement of airway resistance using bodyplethysmograph. Jpn J Physiol 52(suppl): S87, 2002.
6) Bisgaard H, Nielsen KG. Plethysmographic measurements of specific airway resistance in young children. Chest 128: 355-362, 2005.
7) Cantor JO, Cerreta JM, Armand G, *et al*. pulmonary air-space enlargement induced by intratracheal instillment of hyaluronidase and concomitant exposure to 60%oxygen. Exp Lung Res 19: 177-192, 1993.
8) Scuri M, Abraham WM, Botvinnikova Y, *et al*., Hyaluronic acid blocks procine pancreatic elastase (PPE)-induced bronchoconstriction in sheep. Am J Respir Crit Care Med 164: 1-5, 2001.