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Lysozyme hydrochloride Inhibits Cytokines in Epithelial Cells with Respiratory Syncytial Virus Infection: a Brief Report

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ABSTRACT

Lysozyme hydrochloride, derived from Hen Egg Lysozyme, is one of classical anti-airway inflammatory drugs. It has been widely administered in Japan, but its molecular potential is unknown. To confirm this classical drug as anti-inflammatory agent, pro-inflammatory cytokines in Epithelial Cells infected by RSV with lysozyme hydrochloride were measured. Lysozyme hydrochloride suppressed the production of IL-6, IL-1 β , G-CSF although IL-8 and IFN- γ was not changed. Lysozyme hydrochloride inhibits pro-inflammatory cytokines in Epithelial cells infected with Respiratory Syncytial Virus, and may affect to airway inflammation.

Keywords: Lysozyme hydrochloride, Respiratory Syncytial virus, IL-1 β , IL-6, G-CSF.

INTRODUCTION

Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) is very critical, high mortality disease. [1] It is very important in chronic obstructive pulmonary disease (COPD) patients, and airway infection, especially viral infection, is its major cause. [2] There is no definitive management to control airway inflammation induced by viral infection in COPD patients, although many clinicians have very high interests. How do we control airway inflammation and how do we prevent AECOPD? There is nothing conclusive, but many researchers try to find a new agent that has anti-airway inflammatory effect.

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Respiratory syncytial virus (RSV) is known as major cause of young children's airway infection, especially as cause of bronchiolitis. [3] Recently it is realized that this organism may be one of cause of adult bronchitis and bronchiolitis. [4] Therefore it is estimated that this organism is harmful for COPD patient and may be one of cause of AECOPD through airway inflammation. [5] There are some agents to control airway inflammation, clarithromycin [6], L-carbocysteine [7] and tiotropium bromide. [8] These agents suppress cytokines derived from airway inflammatory cells. Lysozyme hydrochloride is commonly administered in Japan as anti-inflammatory agent, especially for chronic airway inflammation. However, its actual molecular potential is still unknown. To confirm whether this classical drug has anti-airway inflammatory effect, we examined the anti-RSV effect of lysozyme hydrochloride by using HEp-2 cells.

MATERIAL AND METHODS

This study is a preclinical study. Institutional Review Board approval was not obtained, since it was not necessary for a basic study.

RSV A2 strain was obtained from ATCC (Rockville, Md., USA). RSV stocks were prepared as described previously.^[9]

HEP-2 cells (CCL-23) were obtained from ATCC (Rockville, Md., USA), and maintained in Eagle's Minimum Essential Media supplemented with glutamine and 10% fetal bovine serum (FBS).

Lysozyme hydrochloride was obtained from Eisai Co., Ltd., Japan.

Preliminary study

A viral titer of supernatants was unchanged 48 hours after infection with RSV at a multiplicity of infection (MOI) of 0.1, and 5ng/ml lysozyme hydrochloride. Cell

viability was not changed with 5ng/ml lysozyme hydrochloride, assessed by ATP assay.

Measurement of cytokines

The effect of lysozyme hydrochloride on cytokine expression after RSV infection was examined with treating the cells 0, 5, 50, 500pg/ml and 5ng/ml lysozyme hydrochloride for 3 days before and 48 hours after RSV infection at an MOI of 0.1. The supernatants were collected and the cytokines were measured by multiplex assay in Bio-Plex assay system (BIO-RAD Laboratories, Inc., Hercules, Ca., USA).

Statistical analysis: Statistical analysis was performed with StatView software (SAS Institute, Cary, N.C., USA). All comparisons to 0pg/ml of lysozyme hydrochloride of means were made by Tukey's test. The level of significance was set at $p < 0.05$.

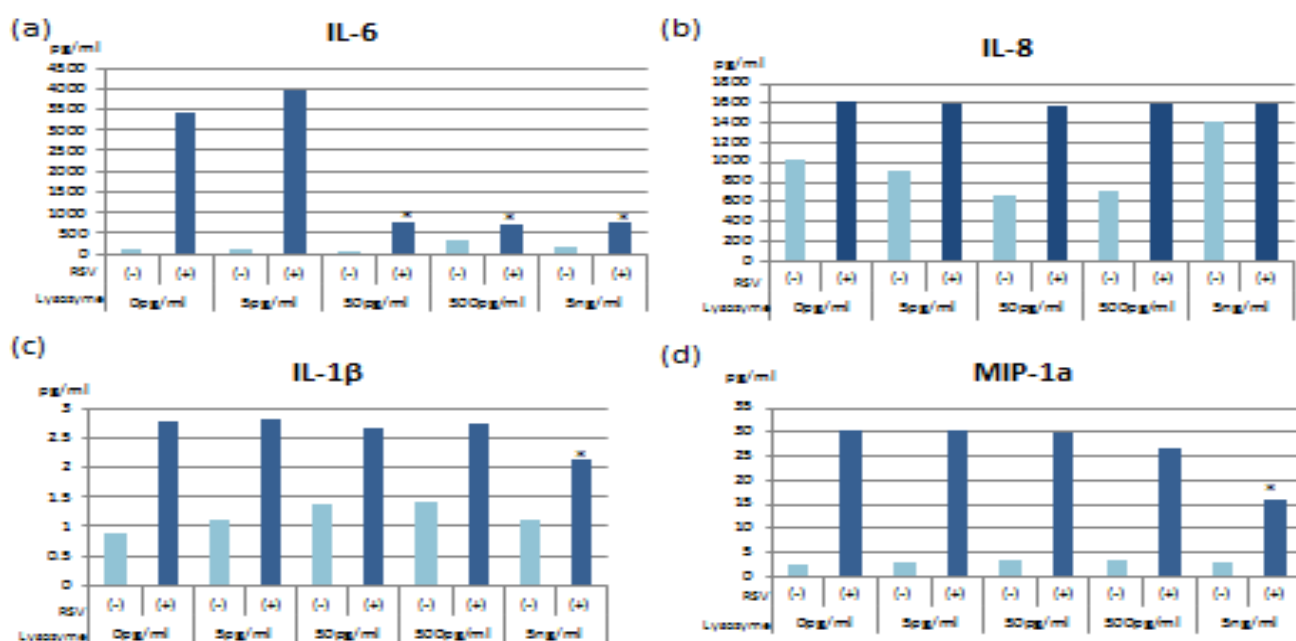


Fig. 1: Effect of Lysozyme hydrochloride on the concentration of IL-6(a), IL-8(b), IL-1β(c), MIP-1α (d). * $p < 0.05$ n=6

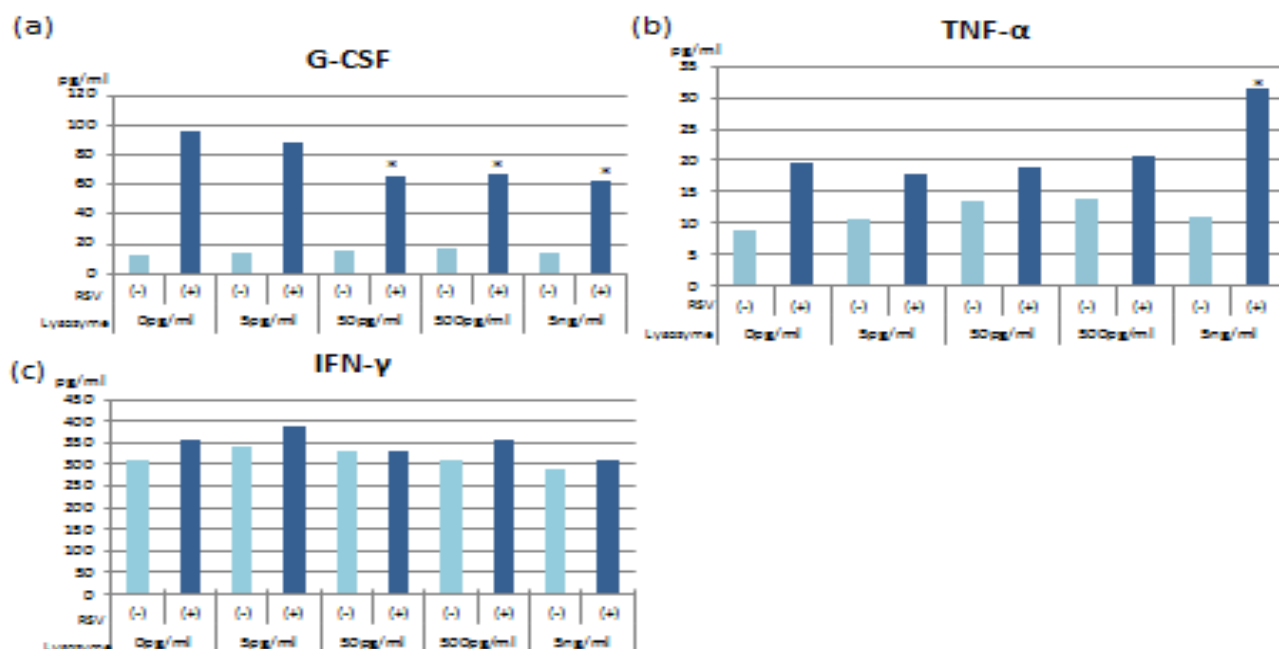


Fig. 2: Effect of Lysozyme hydrochloride on the concentration of IFN-γ (a), TNF-α (b), G-CSF(c). * $p < 0.05$ n=6

RESULTS

The concentration of IL-6 significantly decreased at 50, 500, 5000pg/ml lysozyme hydrochloride, dose dependently, compared with the control (Figure 1a). In other way, they did not show any inhibition to product IL-8 (Figure 1b). IL-1 β and MIP-1a were decreased by lysozyme hydrochloride, at 5,000pg/ml of lysozyme hydrochloride (Figure 1c, d).

G-CSF also decreased as to be the drug concentration (Figure 2a), however, TNF- α increased at 5,000pg/ml of lysozyme hydrochloride (Figure 2b), and, IFN- γ did not show any significant change regardless of the drug concentration (Figure 2c).

DISCUSSION

In the present study, we found that lysozyme hydrochloride could inhibit some of inflammatory cytokines induced by RSV infection in HEp-2 cells. In other way, it was also confirmed that there were cytokines that the agent could not control, e.g. IL-8.

IL-6, IL-8, IL-1 β , IFN- γ and TNF- α are known as important inflammatory cytokines. Our present study showed that IL-6 and IL-1 β were inhibited by lysozyme hydrochloride, though IL-8, IFN- γ and TNF- α were not. The reason why there was discrepancy between them is not declarative, but three reasons were estimated. First, this agent's competence to regulate inflammatory cytokines was partially. Second, more dosing was needed to inhibit inflammatory cytokines. Third, the target pathway of this agent was not standard one, RhoA signaling pathways. In RSV infection, RhoA signaling pathways can induce several cytokines. [10] If lysozyme hydrochloride may affect this pathway, both IL-6 and IL-8 can be regulated.

Partial regulation in our study would make us think that this agent may affect other pathway. We need to make additional and sequential experiments *in vitro* to confirm these points. In the present study, we showed only simple data about major inflammatory cytokines induced by RSV infection in HEp-2 cells. Data of other inflammatory cytokines, viral PCR, plaque assay, measurement cell surface ICAM-1, and RhoA activation are supposed to be needed. It is necessary to demonstrate further investigation. We showed simple data that lysozyme hydrochloride might inhibit some of inflammatory cytokines derived from RSV infection. This simple data is not able to declare lysozyme hydrochloride can control airway infection, and further investigations are needed.

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