Effects of a short-course of pranlukast combined with systemic corticosteroid on acute asthma exacerbation induced by upper respiratory tract infection

Short title: Pranlukast for URI-induced acute asthma

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ABSTRACT

Background: Upper respiratory tract infections (URI) represent the most frequent cause of acute asthma exacerbation. Systemic corticosteroid (CS) is presently recommended for URI-induced asthma exacerbation, although it might inhibit cellular immunity against respiratory virus infection.

Objectives: To determine the effects of adding a short course (two weeks) of a leukotriene receptor antagonist (LTRA) to systemic CS on URI-induced acute asthma exacerbation.

Methods: Twenty-three adult asthmatics (mean age 42.8 ± 9.8 y; M:F, 10:13) with URI-induced acute asthma exacerbation confirmed by a questionnaire and physical findings were randomly assigned to receive either oral prednisolone alone (PSL) or oral PSL plus the LTRA pranlukast (PRL) for two weeks (PSL + PRL). The cumulative doses of PSL and the amount of time required to clear asthma-related symptoms were determined. Levels of respiratory syncytial virus (RSV) RNA and influenza viral (IV) antigen in nasopharyngeal swabs were also determined.

Results: Adding PRL significantly reduced the cumulative dose of PSL and tended to reduce the time required to clear asthma-related symptoms. Either RSV or IV was detected in about one third of the patients.

Conclusions: The combination of an LTRA and CS might be more useful than CS alone for treating URI-induced acute exacerbation of asthma and reducing the cumulative CS dose.

Key words: bronchial asthma, upper respiratory tract infection, leukotriene receptor antagonist, corticosteroid, respiratory syncytial virus

Introduction

Asthma is one of the most prevalent diseases in the world and it has a large socioeconomic impact. The primary objective of therapy for asthma includes not only preventing limitations to routine activities but also reducing the risk of death as along with the economic impact from hospitalization and being absent from work. To date, inhaled corticosteroids (ICS) represent the most effective treatment for asthma and they can reduce mortality due to asthma by preventing acute exacerbation. Global asthma guidelines recommend systemic CS and short-acting β2 agonists (SABA) as soon as acute exacerbation occurs [1]. Viral respiratory tract infections represent the most common trigger of acute exacerbation of asthma in both children and adults [2, 3], and systemic CS might worsen viral infections by suppressing cellular immunity. Furthermore, airway obstruction associated with virus-induced acute exacerbation of asthma is resistant to SABA [4]. Thus, other medications should be added to systemic CS and SABA to treat virus-induced acute asthma exacerbation.

Although the precise underlying mechanism of virus-induced acute exacerbation of asthma remains unknown, many molecular factors and cells are critically involved [5]. Among them, cysteinyl leukotrienes (cysLTs) have received considerable focus because levels increase in the airways of asthmatics during

virus-induced acute exacerbation [6-8] and specific cysLT receptor antagonists (LTRA) are routinely available in clinics. Moreover, systemic CS cannot inhibit cysLT production in asthmatics [9]. Thus, since respiratory viral infections increase the amounts of cysLTs in the airways and CS does not reduce cysLT production, we postulated that LTRA combined with CS might be useful for treating virus-induced asthma exacerbation. The present study compares the effects of short term LTRA plus systemic CS on upper respiratory tract infection (URI)-induced asthma exacerbation with those of CS alone. We defined clinical URI based on symptoms, and determined the presence of respiratory syncytial virus (RSV) and influenza virus (IV) in respiratory secretions.

Materials and methods

Subjects

This study received ethical approval from the special committee of Nagasaki University (project registration number 08090566) to proceed between September 2008 and March 2009 and each patient provided written informed consent to participate. Twenty-three (male:female, 10:13; mean age, 42.8 ± 9.8 y) patients with acute exacerbation of asthma participated in a four-week, randomized, prospective, multi-center trial at outpatient clinics at four institutions in Nagasaki Prefecture, Japan. Eligible individuals were adults with asthma diagnosed according to the GINA guidelines [1] who had received a daily fixed dose of ICS for at least one year before the study. Atopy was defined by a positive skin prick test using 10 common aeroallergens and/or IgE (RAST). We defined clinical URI-induced acute asthma exacerbation as described [10]. We defined URI based on having at least two of the following symptoms: runny nose, stuffy nose, sneezing, sore throat, hoarseness, red or watery eyes, face ache or earache, feeling unwell, muscle aches, chills, cough, painful swollen neck glands or increased use of handkerchiefs. Asthma exacerbation was defined as an increase in one or more of wheeze, chest tightness, and breathlessness or wheeze during clinical examinations. URI-induced acute asthma exacerbation was defined as having symptoms of both URI

and asthma exacerbation. All participants were considered to have URI-induced acute exacerbation of asthma if they attended a hospital within 48 hours of onset. Exclusion criteria comprised being regularly administered with oral CS, LTRA administration within one year before entry, pathogenic bacteria, fungi or acid fast bacilli in expectorated sputum, pulmonary infiltration suggesting pneumonia or requiring hospitalization.

Study design

After obtaining a clinical history regarding URI-induced acute asthma exacerbation, confirming wheezing by a physical examination and their completing a questionnaire, all patients were randomly assigned to receive oral prednisolone (PSL) either without (PSL) or with (PSL + PRL) 225 mg b.i.d. of the LTRA, pranlukast (PRL) (ONON®, ONO Pharmaceutical Co. Ltd., Osaka, Japan) for two weeks. During this period, patients recorded their symptoms and the number of puffs of SABA. All participants had to be taking a stable dose of ICS and patients who used long-acting □2 agonists (LABA) before the study were required to use similar doses of these drugs throughout the study period. Other asthma medications such as xanthines and inhaled anticholinergics were prohibited during the study period. Since a significant number of

patients could not record peak expiratory flow (PEF) within a few days after the first visit due to instability, the present study does not include PEF findings. All of the patients received oral PSL (30 mg/day) during the first four days. Thereafter, each attending physician decided the PSL dose on days 4, 7, 14, 21 and 28 after the first visit based on the following criteria. The PSL dose was reduced by 10 mg when at least two among rescue SABA use, chest auscultation or symptoms were improved compared with the previous assessment. The PSL dose was maintained when one or none of rescue SABA use, chest auscultation and symptoms was improved compared with the previous visit. The PSL was stopped when patients returned to baseline status before exacerbation. Peripheral blood and nasopharyngeal aspirates (NPA) were collected at the first visit and on day 28. The primary and secondary efficacy endpoints were cumulative doses of PSL and elapsed time from the first visit to clear all asthma-related symptoms, respectively. Adverse effects associated with treatment were also monitored throughout the study period.

Diagnosis of respiratory syncytial virus (RSV) and influenza viral (IV) infections

We performed RT-PCR to amplify RSV RNA and detected IV antigen from nasopharyngeal aspirates (NPA). In brief, total RNA was isolated from NPA when the

patients were first examined using TRIzol® (Life Technologies Inc., Rockville, MD, USA). Complementary DNA was synthesized using a SuperScript® One-Step RT-PCR system with Platinum® *Tag* DNA Polymerase (Invitrogen Life Technologies Inc.), and amplified using 200 ng of cDNA, with primers complementary to the sequence of RSV N protein mRNA (sense: 5-GCG ATG TCT AGG TTA GGA AGA A-3, antisense: 5-GCT ATG TCC TTG GGT AGT AAG CCT-3. Influenza viral antigen was determined using an immunochromatographic assay (RapidTesta® Flu II, Sekisui Medical Co. Ltd., Tokyo, Japan). To exclude infection with atypical pathogens, acute and convalescent serum samples were tested for antibodies to *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* (SRL Co., Tokyo, Japan). A fourfold rise in antibody was taken as indicating infection.

Statistics

The intention-to-treat population was defined as randomized patients. The per-protocol population was defined as patients with confirmed assessments available by the end of therapy. Safety was analyzed in the intention-to treat population. Results are expressed as means ± standard deviation (SD). Differences between groups were examined for statistical significance using Mann-Whitney U test and the χ^2 test. A P value < 0.05

denoted the presence of a statistically significant difference.

188 **Results**

189 Enrollment and characteristics of the patients at baseline

190 The 10 and 13 patients assigned to the PSL and PSL + PRL groups, respectively,
191 comprised the intention to treat population. One patient in the PSL group did not record
192 symptoms by day 4, when symptoms and chest wheezes persisted. This patient did not
193 attend the hospital again. One patient in the PSL group and two in the PSL + PRL group
194 never returned to the hospital after initial randomization. Thus, these four patients were
195 not evaluated at the primary and secondary end points, but were recruited for the safety
196 analysis since we could contact them by telephone. Asthma-related symptoms
197 disappeared and PSL was terminated by day 28 in the remaining 19 patients (PSL, n =
198 8; PSL + PRL, n = 11). These 19 patients comprised the per-protocol population. Table
199 1 summarizes the baseline characteristics of the patients in the intention-to-treat
200 population. Baseline demographic characteristics closely matched and no parameters
201 significantly differed between the PSL and PSL + PRL groups. Demographic
202 characteristics also did not notably differ between the intention-to-treat and per-protocol
203 populations (data not shown).

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Duration and cumulative doses of PSL required to eliminate asthma-related symptoms

Figure 1 shows that the durations and cumulative doses of PSL were significantly more decreased in the PSL + PRL, than in the PSL group (Figure 1) (7.3 ± 4.5 vs. 14.0 ± 7.7 days, $p = 0.03$ and 169.1 ± 62.8 vs. 253.8 ± 86.0 mg, $p = 0.03$, respectively). In contrast, cumulative doses of SABA were similar between the PSL and PSL + PRL groups (14.0 ± 3.2 vs. 11.5 ± 3.4 puffs, $p > 0.1$).

Time required to eliminate asthma-related symptoms

Adding PRL tended to shorten exacerbations compared with PSL alone, but the difference did not reach statistical significance (Figure 2) (PSL vs. PSL + PRL: 16.4 ± 6.7 vs. 10.7 ± 5.9 days, $p = 0.06$).

Virus detection

Either RSV or IV was detected in 6 (31.6%) patients. Three (PSL, n = 2; PSL + PRL, n = 1) were positive for RSV according to RT-PCR. Influenza viral antigen was detected in three patients (PSL, n = 1; PSL + PRL, n = 2). Asthma-related symptoms disappeared from the three patients infected with RSV at days 27, 21 and 10 and from the three

infected with IV infected on days 14, 7 and 6. Significant differences in serum antibodies titers of *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* were not identified between acute and convalescent phases, suggesting that atypical pathogens were absent.

Safety

Safety was evaluated in the intention-to-treat population. Among 23 patients, 4 (17.4%) experienced at least one adverse event during the study period. One patient in the PSL group developed headache, epigastralgia and insomnia and another developed epigastralgia. One patient in the PSL + PRL group developed epigastralgia and another described having diarrhea. Severe infectious diseases did not occur. All adverse events were clinically mild and had subsided by the end of the study. Thus the incidence of adverse events did not significantly differ between the two groups.

Discussion

Although LTRAs reduce asthma symptoms or exacerbations in children with colds [11-13], few studies have evaluated their effects on asthma in adults. One study found that LTRAs do not improve symptoms of mild asthma caused by experimental rhinovirus infection in adults [14]. Thus the role of LTRA in acute asthma exacerbation in adults caused by naturally occurring viral respiratory infection remains unknown. The present study showed that combining the LTRA, pranlukast, with systemic CS for 2 weeks slightly shortened the duration of asthma-related symptoms and significantly reduced cumulative CS doses in adult patients who developed acute asthma exacerbation after upper respiratory tract infection.

Viral respiratory tract infections often exacerbate asthma, which can be significantly reduced by the regular administration of inhaled CS [15]. In contrast, systemic CS significantly increased viral loads in healthy individuals after experimental RV infection [16] and reactivated chronic metapneumoviral infection in a murine model [17]. Since systemic CSs are recommended for acute exacerbation of asthma even by respiratory viral infection [1], the notion that they might suppress immunity against respiratory virus should be a concern. Thus, an additive therapy that would increase the beneficial effects and decrease the toxicity of systemic CS would be useful. From this

viewpoint, cysLTs are appealing because their concentrations increase during respiratory virus-induced asthma [6-8] and CSs cannot inhibit their production [9]. In fact, the LTRA montelukast prevents respiratory virus-induced acute asthma in children [11-13]. We also reported that pranlukast inhibits RSV-induced allergic airway inflammation in a murine model of allergic asthma [18]. Currently, LTRAs are used as controlling, anti-inflammatory medication. Notably, LTRAs have bronchodilator as well as anti-inflammatory effects and a rapid onset of action. Thus, LTRAs are potentially useful to relieve acute asthma [19, 20].

Respiratory syncytial virus is a representative lower respiratory tract pathogen in children that has recently become recognized as an adult pathogen [21-23]. One study in vitro has demonstrated that RSV enhances 5-lipoxygenase expression in the human airway epithelium and thus increases LT production [24], which potentially exacerbates allergic airway inflammation. Furthermore, RSV among respiratory viruses causes acute asthma exacerbation more frequently than influenza virus [25]. Although we identified a few causative viruses, the present study found that RSV actually causes acute asthma exacerbation in adults. Although the study cohort was too small to be statistically meaningful, the results suggested that acutely exacerbated asthma takes longer to improve when caused by RSV rather than by IV.

Besides LTRA, LABA also has anti-viral as well as bronchodilator effects [26]. Probably due to the small number of patients (n = 6), the use of LABA before entry did not significantly affect the present results (data not shown). A future study should also examine the effects of adding LABA in URI-induced acute exacerbation of asthma.

The present study has several critical limitations. Firstly, we defined URI based on clinical symptoms and only RSV and IV were detected. Although bacterial and other infections were excluded by physical and laboratory examinations, non-viral infections might have been included. Secondly, asthma-related symptoms were also evaluated based on clinical symptoms and objective measures of pulmonary functions such as PEF or FEV1.0 were missing because the patients had unstable asthma. Finally, this was not a placebo-controlled study and the cohort was small.

Conclusions

The present findings suggest a new therapeutic modality for LTRAs as a means of controlling chronic asthma. As in intermittent childhood asthma [15], LTRAs could be combined with CS only to relieve acute asthma induced by URI. Placebo-controlled, large scale studies including many types of representative respiratory viruses are warranted.

295

296 None of the authors have conflicts of interest to claim other than a grant from ONO

297 Pharmaceutical Co. Ltd. to conduct the study.

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Table 1. Characteristics of the intention-to-treat population.

Characteristics	PSL	PSL + PRL
N	10	13
Age* (y)	48.2 (12.9)	41.9 (11.7)
Gender, M (F)	5 (5)	5 (8)
Atopy (%)	40.0	38.5
Disease duration* (y)	23 (5.4)	28 (6.7)
Maintenance ICS	375.0 (195.6)	368.8 (164.2)
FP equivalent* (μg/day)		
LABA use (%)	20.0	30.8
Time from onset of symptoms to first assessment* (days)		
	4.1 (2.1)	3.7 (1.9)

* Values are shown as means (SD).

Figure legends

Figure 1. Time using PSL (upper) and cumulative doses of PSL (lower) to eliminate asthma-related symptoms in the per-protocol population.

Bars represent means ± SD of PSL (n = 8) and PSL + PRL (n = 11) groups; *$p < 0.05$.

Figure 2. Time required to clear asthma-related symptoms in the per-protocol population.

Bars represent means ± SD of PSL (n = 8) and PSL + PRL (n = 11) groups. †$p < 0.1$.

Figure 1

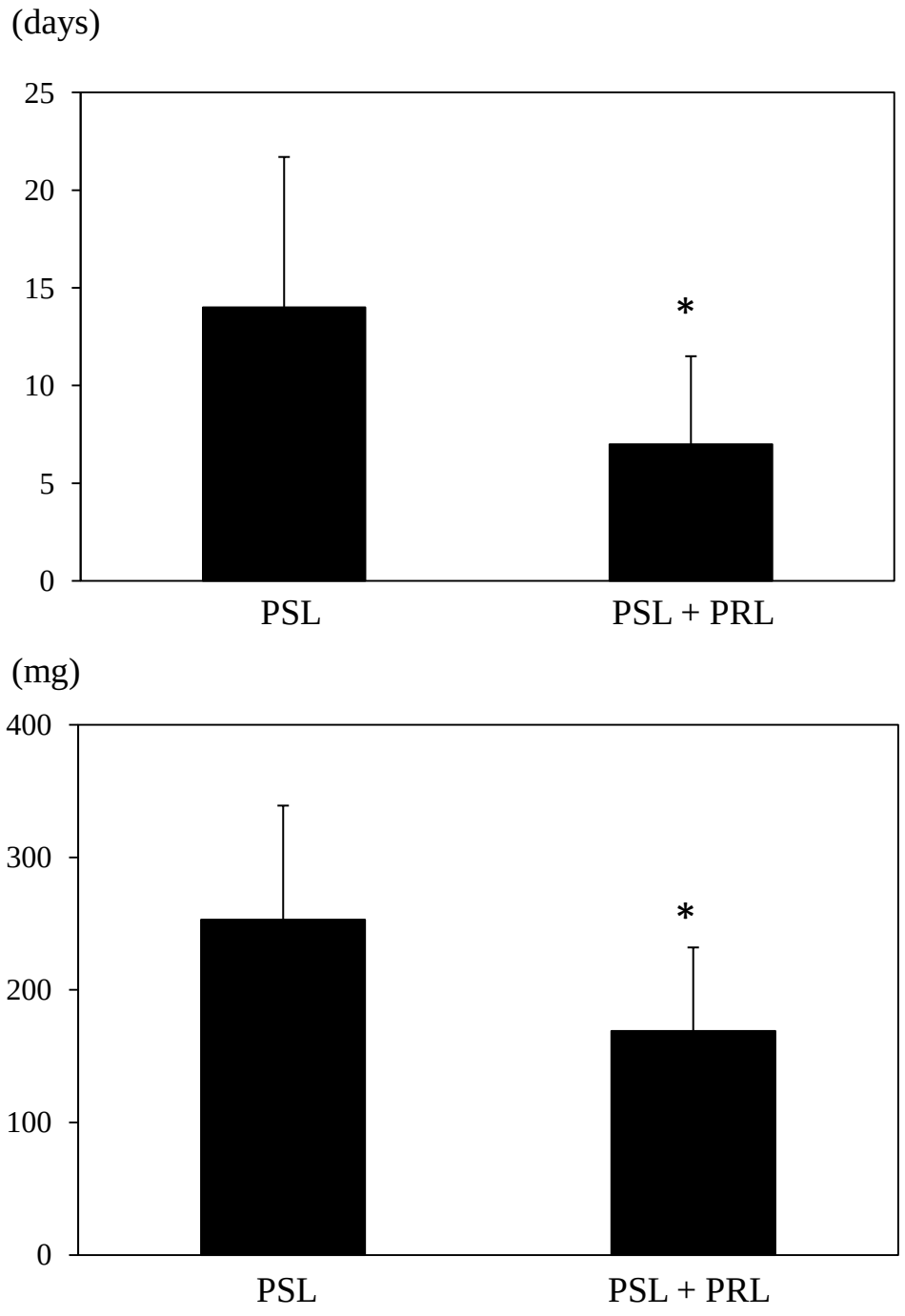

Figure 2