

Anti-inflammatory effect of roxithromycin in patients with aspirin-intolerant asthma

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Summary

Background Fourteen-membered macrolides, such as roxithromycin, have been reported to exhibit other pharmacological activity including anti-asthmatic effects, besides antibiotic activity.

Objective This study was designed to investigate the protective effect of roxithromycin on airway responsiveness to the sulphuric acid provocation test and to investigate whether this protective activity is associated with a reduction in aspirin-induced excretion of urinary leucotriene E₄ (u-LTE₄), a marker of cysteinyl leucotriene overproduction that participates in the pathogenesis of aspirin-intolerant asthma. Also, the present study was designed to examine whether or not its anti-asthmatic activity was associated with a reduction in eosinophilic inflammation.

Methods For 8 weeks before analysis, subjects received 150 mg of roxithromycin or matching placebo twice daily. We assessed the effects of pretreatment with roxithromycin on bronchoconstriction precipitated by inhalation of sulphuric acid in 14 adult patients with mild or moderate aspirin-intolerant asthma; those who were in stable clinical condition and were hyperresponsive to sulphuric acid provocation test were allocated to this study. A double-blind, randomized, crossover design was used. Urinary LTE₄ was measured by a combined reverse-phase high-performance liquid chromatography (rp-HPLC) enzyme immunoassay on sulphuric acid provocation testing day. Blood and sputum samples were taken in the morning on the sulphuric acid provocation testing day. Eosinophil counting and measurement of eosinophilic cationic protein (ECP) were performed.

Results After the 8 weeks of treatment with roxithromycin, patients' symptoms, blood eosinophils, serum ECP, sputum eosinophils, and sputum ECP were significantly decreased. On the other hand, values of PC₂₀-sulphuric acid did not improve after roxithromycin at all. Furthermore, although challenge with sulphuric acid caused a significant increase in u-LTE₄, pretreatment with roxithromycin or placebo did not affect excretion of u-LTE₄.

Conclusion Although roxithromycin does not have antileucotriene effects, it has an antibronchial inflammatory effect associated with eosinophilic infiltration. This study raises further interesting therapeutic possibilities and warrants further trials of new approaches to the treatment of aspirin-intolerant asthma.

Keywords: aspirin-intolerant asthma, bronchial hyperresponsiveness, bronchial inflammation, eosinophils, roxithromycin

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Introduction

The prevalence of aspirin-intolerant asthma is close to 20% in the general asthmatic population [1–3]. In the subset of asthmatics with nasal polyps and sinusitis, it was shown to be 34% of those asthmatics prospectively challenged with acetylsalicylic acid [3]. Although aspirin-intolerant asthma has been widely recognized as a clinical entity since the beginning of this century, the exact pathological mechanism for the production of this syndrome still remains obscure. Some possible mechanisms have been proposed for aspirin-intolerant asthma, such as bronchial overproduction of leucotrienes and inhibition of prostaglandin (PG) synthesis [4–6], overproduction of bronchoconstrictive PGs such as PGD₂ metabolite 9 α , and 11 b -PGF₂ [7,8], and some other hypotheses may be involved in the pathogenesis of aspirin-intolerant asthma.

Roxithromycin, a fourteen-membered macrolide, has attracted attention for its effectiveness in a variety of respiratory diseases, including bronchial asthma [9]. We assessed the effects of pretreatment with roxithromycin on bronchoconstriction precipitated by inhalation of sulpyrine in aspirin-intolerant asthma, because the effect of 14-membered macrolides against aspirin-intolerant asthma has never been reported in double-blind randomized crossover studies.

This study was designed to investigate the protective effect of roxithromycin on airway responsiveness to the

sulpyrine provocation test and to investigate whether this protective activity is associated with a reduction in aspirin-induced excretion of urinary leucotriene E₄ (u-LTE₄), a marker of cysteinyl leucotriene overproduction that participates in the pathogenesis of aspirin-intolerant asthma. Also, the present study was designed to examine whether or not its anti-asthmatic activity was associated with a reduction in eosinophilic inflammation.

Methods

Subjects

We studied 14 adult patients with clinical histories of aspirin-intolerant asthma. Sulpyrine and/or lysine aspirin provocation test were positive in all of them. All patients were non-smokers and diagnosed with mild or moderate asthma according to American Thoracic Society criteria [10]. The patients were in stable condition and had been free of symptoms of respiratory infection for at least 6 weeks (Table 1).

Patients using oral or inhaled corticosteroids, theophylline, any antileucotriene drug, such as pranlukast, or any other anti-allergic agents as well as roxithromycin were excluded. All patients used a metered-dose inhaler of β -stimulants to control their asthma. The study was conducted according to the ethical standards of our hospitals, which require informed consent from each patient.

Table 1. Patient characteristics at time of entry into the study. Sulpyrine and/or lysine aspirin provocation tests were all positive. All patients were diagnosed with mild or moderate asthma according to American Thoracic Society's criteria. The patients were in stable condition and had been free of symptoms of respiratory infections for at least 6 weeks.

Patient	Sex	Age (years)	Atopy	FEV ₁ (% pred.)	FVC (% pred.)	Eosinophils (%)	IgE (RIST) (U/mL)	PC20-Sulpyrine (mg/mL)	
								Placebo	Roxithromycin
1	M	29	HD, Gr	76	97	9	466	10	10
2	M	42	Cd	70	96	4	132	10	20
3	M	35	HD, C, Gr	87	103	7	475	10	10
4	M	39	None	84	94	5	151	20	10
5	M	40	None	72	96	3	54	20	20
6	M	45	Cd	76	99	8	260	10	4
7	F	42	Gr, HD	70	97	5	133	10	10
8	F	29	C	76	89	4	37	20	20
9	F	38	None	70	91	6	116	10	10
10	F	46	Cd	69	101	4	245	20	20
11	F	30	None	88	88	4	89	250	250
12	F	50	None	70	97	6	63	10	10
13	F	42	Hd, Cd	74	118	8	340	20	20
14	F	47	None	68	82	10	77	4	4

HD, house dust; C, cat; Gr; grass; Cd, candida. All patients used a metered dose inhaler of β -stimulants to control their asthma.

Study design

Subjects were randomly assigned to receive an 8-week course of two daily doses of 150 mg of roxithromycin (Eisai, Osaka, Japan) or a placebo dose identical in appearance. After a washout period of at least 4 weeks, the subjects crossed over to receive the alternative treatment for 8 weeks.

FEV₁ and FVC were measured and blood and intolerant sputum differential and total cell counts were determined before and after each 8-week treatment course.

Blood count and serum eosinophilic cationic protein (ECP) measurement

Blood samples for eosinophil counting and measurement of ECP were taken between 08.00 and 09.00 h on the same day before sulpyrine provocation testing. Blood was collected into heparinized tubes by direct venipuncture, and blood smears were made for differential staining (Diff quick; American Scientific Products, McGaw Park, IL, USA). Differential cell counts were calculated as the mean value of two slides, with 300 cells counted per slide. The total leucocyte count was determined with a haemocytometer (Neubauer Chamber; Hausser Scientific, Blue Bell, PA, USA), and cell populations were expressed as number of cells millilitre of blood.

To avoid interassay variations, all ECP measurements were performed in the same run. Blood (4 mL) was collected into on SST tube (Becton Dickinson, Mountain View, CA, USA), stored for 60 min at 25 °C, and subsequently centrifuged at 13 000 *g* for 10 min at 4 °C. Serum was stored at –80 °C until ECP levels were measured with an ECP RIA kit (Pharmacia Diagnostics, Uppsala, Sweden). The detection limit of this assay is 2.0 µg/L.

Sputum analysis

Sputum was induced and processed as described by Popov and colleagues [11]. Subjects inhaled 3, 4 and 5% saline for 7 min each. The induction was stopped when an adequate sample was obtained, or if the FEV₁ dropped $\geq$ 20% from baseline. Cell plugs with few or no squamous epithelial cells were selected from the sample with an inverted microscope, separated from saliva and weighed. Samples were aspirated in two times their volume of 0.1% dithiothreitol (Sputolysin, Calbiochem Corp., San Diego, CA, USA) and two times their volume of Dulbecco's phosphate-buffered saline (DPBS; Gibco, Grand Island, NY, USA). The cell suspension was filtered through nylon gauze (mesh size is 52 µm; BNSH Thompson, Scarborough, ON, Canada) to remove debris, then centrifuged at 1300 *g* for 10 min at 4 °C. Supernatants were collected and stored at –80 °C for fluid-phase measurements of ECP.

The total cell count was determined with a haemocytometer (Neubauer Chamber) and expressed as the number of cells per millilitre of sputum. Cells were resuspended in DPBS at a concentration of $0.75\text{--}1.0 \times 10^6/\text{mL}$. Cell preparations for cytocentrifusion were made on glass slides from 50 µL of cell suspension and subjected to cytocentrifuge (Shandon III, Shandon Southern Instruments, Sewickey, PA, USA), at 333 *g* for 5 min. Differential cell counts were obtained from the mean of two slides stained with Diff Quick with 400 cells counted per slide. Cell types were quantified as number of cells per millilitre of sputum. Cell preparations for cytocentrifusion were made on apex-coated slides and fixed for 10 min in periodatylsine-paraformaldehyde for immunocytochemical staining for ECP.

Sulpyrine provocation testing

Sulpyrine inhibits cyclo-oxygenase and thus may share a common mechanism of action with aspirin, though its inhibitory activity is less potent than that of aspirin [12]. The sulpyrine provocation test is now widely used for diagnosis of aspirin-intolerant asthma in Japan. Bronchial challenge with sulpyrine was performed according to the standard method of Japanese Allergology Society as previously described [13]. Fresh pulverized sulpyrine (Astra Japan, Inc., Osaka, Japan) was dissolved each day in saline to produce reagents. This test was performed using a jet nebulizer driven by compressed air (Nihon Shoji Co. Ltd, Osaka, Japan).

All patients were given a provocation test after each 8-week treatment course with either roxithromycin or placebo in a double-blind, randomized manner. Thereafter subjects were crossed over to receive alternative treatment and followed identical procedure at the same time of day. Each study day began with measurement of FEV₁ and FVC for baseline and then inhalation of nebulized saline to rule out a non-specific obstructive reaction; FEV₁ was measured 5 min before and 5, 10 and 15 min after completion of inhalation. If FEV₁ did not fall more than 10%, the subject entered the main part of study. The sulpyrine provocation test was performed at concentrations as follows; 10, 20, 100, 250, 500 and 1250 mg/mL for all but one subject who was too sensitive to challenge at these doses of sulpyrine, and so was given 4 and 10 mg/mL. These concentrations are in accordance with our daily protocol of sulpyrine in an inhalation test to diagnose aspirin-intolerant asthma.

Spirometry was performed again and recorded at 5, 10, 15 and 30 min after inhalation. The provocation test was terminated by the administration of an inhaled bronchodilator if FEV₁ fell by more than 35% from baseline. The higher of the first two satisfactory recordings was taken for analysis. If FEV₁, measured after the inhalation at the time points mentioned above, did not drop by more than 10% of

the initial values, a higher concentration of sulpyrine was given. The provocation was stopped when FEV₁ fell to 20% or more from the baseline or when the maximum dose of sulpyrine was reached. Dose-response relations for sulpyrine were constructed and used for calculation of provocative concentration causing a 20% fall in FEV₁ on sulpyrine inhalation testing (PC₂₀-sulpyrine). For statistical evaluation, their PC₂₀-sulpyrine values were set as equal to the highest concentration of sulpyrine given.

Measurement of urinary LTE₄

Urine was collected before pretreatment with roxithromycin or placebo, and at 2- and 4-h intervals thereafter and stored at -80 °C until assay.

LTE₄ was measured by a combined rp-HPLC (Class LC-10 system: Shimadzu, Kyoto, Japan) enzyme immunoassay as previously described [14]. ³H-LTE₄ (168.4 Ci/mmol; NEN, DuPont, Dreierich, Germany) was used as the internal standard; antibody to LTE₄ (Cayman Chem, Ann Arbor, MI, USA) showed cross-reactivity 10–25% vs LTC₄-LTD₄ and <0.01% vs LTB₄. Creatinine concentration was measured using a standard assay kit (Sigma, St Louis, MO, USA); results, corrected according to the observed recovery, were expressed as the amount of LTE₄ per milligram of creatinine.

Symptom score

To evaluate the effect of roxithromycin on the improvement of symptoms in asthmatics, symptoms were assessed

weekly by physicians using 4 grades: 0 = asymptomatic more than 4 days/week; 1 = mild attack (aware of symptoms but easily tolerated) more than 4 days/week and nocturnal asthma symptoms less than 2 days/week; 2 = moderate attack (discomfort enough to interfere with daily activity) more than 4 days/week and/or nocturnal asthma symptoms more than 2 days/week; and 3 = severe attack (unable to perform usual activity) more than 4 days/week and/or nocturnal asthma symptoms almost everyday. This method is modelled after that of Oosaki and colleagues [15].

Statistical analysis

Statistical evaluation employed StatView J-4.02 for Macintosh (Abacus Concepts, Inc., Berkeley, CA, USA). All PC₂₀ values were logarithmically transformed before analysis, and summary statistics were expressed as mean ± standard deviation (SD). Student's paired *t*-test was used to compare mean differences. Wilcoxon's signed rank test was used for comparison of the two treatment groups at the same time point regarding FEV₁ and u-LTE₄ values. A *P*-value ≤ 0.05 (two-sided) was considered significant.

Results

All subjects could be followed for longer than 120 min. FVC was not significantly affected by administration of roxithromycin or placebo before each provocation test, but FEV₁ did not increase significantly after 8 weeks' treatment with roxithromycin (2.37 ± 0.30 after roxithromycin vs

Table 2. Comparison of parameters measured before and after 8 weeks' treatment with roxithromycin in patients with bronchial asthma.

Parameter	Mean ± SD of indicated parameter		
	Before treatment†	After placebo	After roxithromycin
FEV ₁	2.26 ± 0.22	2.25 ± 0.26	2.37 ± 0.30
FVC	3.19 ± 0.48	3.24 ± 0.42	3.17 ± 0.43
<i>Blood</i>			
Eosinophils (×10 ⁴ /mL)	43.3 ± 7.3	42.8 ± 7.6	12.4 ± 2.3**
Neutrophils (×10 ⁴ /mL)	452 ± 47	448 ± 51	417 ± 72
Serum ECP (mg/L)	15.8 ± 6.3	14.8 ± 7.6	3.6 ± 1.4*
<i>Sputum</i>			
Eosinophils (×10 ⁴ /mL sputum)	94 ± 28	90 ± 33	10 ± 6*
Neutrophils (×10 ⁴ /mL sputum)	271 ± 69	253 ± 72	197 ± 80
ECP (mg/L sputum)	1.8 ± 0.4	1.7 ± 0.9	0.4 ± 0.1*
Symptom score	1.60 ± 0.24	1.63 ± 0.48	0.87 ± 0.70*

All values marked with asterisks were significantly greater than baseline: **P* < 0.05, ***P* < 0.01. †Baseline.

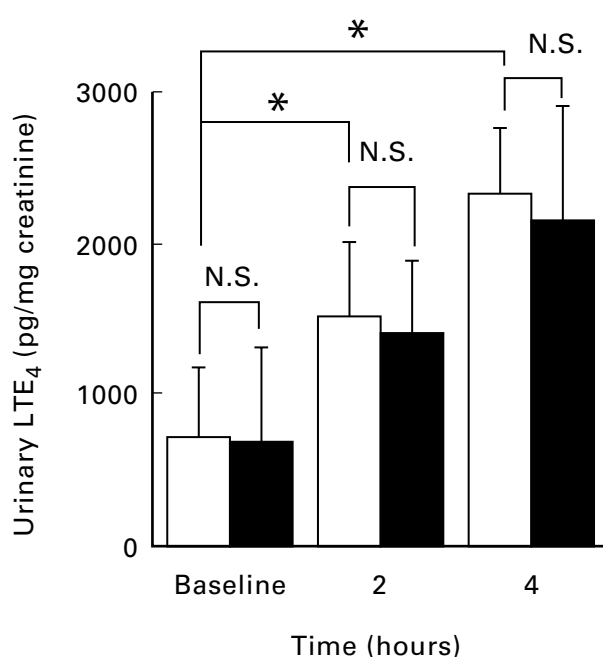


Fig. 1. Mean urinary LTE₄ before and during the observation period after placebo (white bars) or roxithromycin (black bars). Asterisks indicate statistically significant difference: * $P < 0.05$. NS, not significant.

2.25 ± 0.26 after placebo, not significant). Thus, we did not confirm the effect of bronchodilation in long-term treatment with roxithromycin in this study.

After the 8 weeks of treatment with roxithromycin, blood eosinophils, serum ECP, sputum eosinophils, and sputum ECP were significantly decreased (Table 2). On the other hand, the number of blood and sputum neutrophils did not change significantly.

The symptom score significantly decreased after treatment with roxithromycin (1.63 ± 0.48 after roxithromycin vs 0.87 ± 0.70 after placebo, $P < 0.05$). Twelve of the 14 subjects improved their general symptoms, such as nocturnal cough, wheezing, severity or frequency of asthmatic attack. Other subjects felt no change in their symptoms, since their conditions were stable. No subject got worse than before the beginning of this study.

After the pretreatment of roxithromycin or placebo, challenge with sulpyrine caused an obstructive reaction in all patients. PC₂₀-sulpyrine data for all patients are displayed in Table 1. In the provocation challenge with sulpyrine, values of PC₂₀-sulpyrine did not improve after roxithromycin at all. Mean log PC₂₀-sulpyrine was 1.18 ± 0.40 after roxithromycin, vs 1.15 ± 0.43 after placebo (not significant; data not shown).

Although challenge with sulpyrine caused a significant increase in u-LTE₄, pretreatment with roxithromycin or placebo did not affect excretion of u-LTE₄ (Fig. 1).

None of the subjects reported any adverse effect after roxithromycin administration, nor could they discriminate between roxithromycin and placebo.

Discussion

The inhibitory effect of acetylsalicylic acid on the cyclo-oxygenase increases bronchial expression of LTC₄ synthase, allowing marked overproduction of cysteinyl leucotrienes leading to bronchoconstriction [16]. They indicated that long-term overproduction of cysteinyl leucotrienes in aspirin-intolerant asthma patients has been linked to airway eosinophilia. The severity and rapidity of bronchospasm, induced by cyclo-oxygenase-inhibiting drugs in aspirin-intolerant asthma, is directly related to the dose and potency of the drug to inhibit the cyclo-oxygenase. Knapp and colleagues showed that excretion of u-LTE₄ was increased during aspirin-intolerant asthma episodes, but the degree of increase for individual patients did not correlate with the degree of bronchospasm or inhibition of platelet thromboxane B₂ formation [17]. The results indicate that although the endogenous synthesis of potent bronchoconstrictor leucotrienes increases during aspirin-induced bronchospasm, it is unlikely that a direct shunting of unmetabolized arachidonate into leucotriene synthesis represents the mechanism of aspirin-intolerant asthma. Nevertheless, Yoshida and colleagues showed that cromolyn sodium, a well established anti-inflammatory drug for the treatment of asthma, suppressed u-LTE₄ excretion in aspirin-intolerant asthma [18]. Cromolyn sodium inhibits u-LTE₄ excretion and improved PC₂₀-sulpyrine, but challenge with sulpyrine caused an obstructive reaction in all patients. This result suggests that some factors other than cysteinyl leucotriene overproduction should be involved in the pathogenesis of aspirin-intolerant asthma. However, 8-week course of treatment with roxithromycin did not suppress u-LTE₄ excretion in aspirin-intolerant asthma, though the treatment decreased eosinophils and ECP. This finding is not consistent with the results by Cowburn *et al.*

Recently, it has been demonstrated that airway hyper-responsiveness is a characteristic of bronchial asthma, and it has been emphasized that airway inflammation plays an important role in bronchial hyperresponsiveness in patients with bronchial asthma [19,20]. Bronchial inflammation associated with eosinophilic infiltration is especially thought to be involved in bronchial hyperresponsiveness. It has also been reported that, after bronchial instillation of aspirin, biopsies showed a decrease in the number of mast cells and activation of eosinophils [7]. These findings

suggested that activation of eosinophils and degranulation of mast cells are early events associated with aspirin-intolerant reactions. Szczeklik and colleagues reported a statistically significant rise in peptidoleucotrienes, IL-5 and eosinophils in bronchoalveolar lavage of patients with aspirin-intolerant asthma after bronchial challenge with aspirin.

Fourteen-membered macrolides, such as roxithromycin, have been reported to exhibit other pharmacological activity including anti-asthmatic effects, besides antibiotic activity. Miyatake and colleagues demonstrated that erythromycin significantly improves bronchial hyperresponsiveness in both atopic and nonatopic asthma [9].

Serum ECP is thought to be a useful marker of eosinophilic inflammation in bronchial asthma [21]. In this study, 8 weeks' treatment with roxithromycin for patients with asthma improved donors' symptom scores and decreased both blood and sputum ECP and the number of eosinophils. These results confirm that roxithromycin not only has antibiotic activity, but also a significant anti-eosinophilic effect. Adachi and colleagues reported that treatment with theophylline, glucocorticoids, roxithromycin and other macrolides induced apoptosis in guinea pig eosinophils [22]. Konno and colleagues showed that roxithromycin inhibited the appearance of IL-3, IL-4, IL-5, and TNF- α in mouse lung extracts [23]. These properties indicate that 14-membered macrolides may inhibit eosinophilic functions and has immunoregulatory effects. Our results clinically support their investigations.

In conclusion, roxithromycin not only has an antibacterial activity, but also an anti-inflammatory effect associated with eosinophilic infiltration in patients with aspirin-intolerant asthma, although roxithromycin did not inhibit bronchoconstriction and u-LTE₄ excretion on sulpyrine provocation testing. Furthermore, this is the first report to suggest that not only leucotriene overproduction but also eosinophilic bronchial inflammation is involved in the pathogenesis of aspirin-intolerant asthma.

This study raises further interesting therapeutic possibilities and warrants further trials of new approaches to the treatment of aspirin-intolerant asthma.

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