

Effect of acyclovir on bronchoconstriction and urinary leukotriene E₄ excretion in aspirin-induced asthma

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Background: Acyclovir (9-[2-hydroxyethoxymethyl] guanine), an inhibitor of the DNA polymerase of the herpes virus, has been reported to exhibit pharmacologic activity other than antiviral activity, including antiasthmatic effects.

Objective: This study was designed to investigate the protective effect of acyclovir on airway responsiveness to the sulphuric provocation test and to investigate whether this protective activity is associated with a reduction in aspirin-induced excretion of urinary leukotriene E₄ (u-LTE₄), a marker of cysteinyl leukotriene (LT) overproduction that participates in the pathogenesis of aspirin-induced asthma.

Methods: We assessed the effects of pretreatment with acyclovir on bronchoconstriction precipitated by inhalation of sulphuric in 16 adult patients with mild or moderate aspirin-induced asthma; those who were in stable clinical condition and were hyperresponsive to the sulphuric provocation test were allocated to this study. A double-blind, randomized, cross-over design was used. u-LTE₄ was measured by a combined reverse-phase HPLC enzyme immunoassay.

Results: Acyclovir protects against aspirin-induced attacks of asthma through mechanisms unrelated to its bronchodilator property but related to the improvement of bronchial hyperresponsivity to sulphuric; protection was nearly complete in all patients ($P < .0001$). By contrast, after acyclovir, the maximum level of u-LTE₄ in patients was significantly lower than that in control subjects ($P < .01$).

Conclusion: Our results suggest that acyclovir is not only an antiviral drug but also an inhibitor of analgesic-induced bronchoconstriction, probably acting by inhibiting the release of cysteinyl LTs. (*J Allergy Clin Immunol* 1998;102:909-14.)

Key words: Acyclovir, aspirin-induced asthma, cyclooxygenase, leukotriene.

Abbreviations used

FVC: Forced vital capacity
LT: Leukotriene
PG: Prostaglandin
u-LTE₄: Urinary leukotriene E₄

Acyclovir (9-[2-hydroxyethoxymethyl] guanine) is an antiviral drug especially effective in herpes virus infection.¹ The antiviral effect of acyclovir is probably achieved by inhibition of the DNA polymerase of the herpes virus. Acyclovir has been reported to exhibit several pharmacologic functions other than antiviral activity, such as an antiasthmatic effect,²⁻⁴ an antirheumatic effect,⁵ and efficacy in improving aplastic anemia.^{6,7} In particular, some reports indicate that acyclovir reduces hypersensitivity to acetylsalicylic acid or other nonsteroidal antiinflammatory agents in patients with aspirin-induced asthma.^{2,3}

The prevalence of aspirin-induced asthma is close to 20% in the general asthmatic population,⁸⁻¹⁰ and in the subset of asthmatic subjects with nasal polyps and sinusitis, its prevalence was shown to be 34% of those subjects prospectively challenged with acetylsalicylic acid.¹⁰ Although aspirin-induced asthma has been widely recognized as a clinical entity since the beginning of this century, the exact pathologic mechanism for the production of this syndrome still remains obscure. Some possible mechanisms have been proposed for aspirin-induced asthma, such as bronchial overproduction of leukotrienes (LTs) and inhibition of prostaglandin (PG) synthesis.¹¹⁻¹³ Overproduction of bronchoconstrictive PGs (eg, PGD₂ metabolite 9 α and 11 β -PGF₂)^{14,15} chronic viral infection,¹⁶ and some other factors have also been hypothesized to be involved in the pathogenesis of aspirin-induced asthma.

On the other hand, current evidence indicates that cyclooxygenase inhibition and increased production of cysteinyl LTs play important roles in these obstructive reactions. Aspirin sensitivity has disappeared in some patients with aspirin-induced asthma during acyclovir treatment for herpes simplex infection.^{2,3}

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TABLE I. Baseline characteristics of 16 patients in a study of the effects of acyclovir in patients with aspirin-induced asthma

Patient no	Sex	Age (y)	Atopy	FEV ₁ (% predicted)	FVC (%)	Eosinophils (%)	IgE (RIST) (U/mL)	Medication
1	M	36	HD, Gr	66	98	8	480	T, B
2	M	32	Cd	60	96	4	118	IS, T, B
3	M	20	HD, C, Gr	77	120	7	560	IS, B
4	M	45	None	54	92	5	149	T, B
5	M	52	None	66	96	2	222	IS, B
6	M	44	None	72	94	3	106	IS, B
7	M	40	Cd	66	98	8	210	IS, B
8	F	38	Gr, HD	70	100	5	153	T, B
9	F	32	C	76	88	4	48	B
10	F	36	None	60	92	6	320	IS, B
11	F	26	Cd	61	104	4	410	IS, B
12	F	48	None	67	94	5	120	T, B
13	F	40	None	68	84	4	115	T, B
14	F	42	None	52	96	6	89	IS, T, B
15	F	54	HD, Cd	74	121	10	620	T, B
16	F	56	None	51	79	11	60	T, B

HD, House dust; C, cat; Gr, grass; Cd, Candida; T, theophylline; B, inhaled β_2 -agonist on demand; IS, inhaled steroids.

The aim of this study is to confirm previous observations of the protective activity of acyclovir against aspirin-induced asthma and to investigate the mechanism of this protective effect. This study was also designed to examine whether this protective activity is associated with a reduction in sulpyrine-induced urinary LTE₄ (u-LTE₄), a marker of the cysteinyl LT overproduction that participates in the pathogenesis of aspirin-induced asthma.

METHODS

Subjects

We studied 16 adult patients with clinical histories of aspirin-induced asthma diagnosed with mild or moderate asthma according to both the Japanese Allergy Society's criteria and the American Thoracic Society's criteria.¹⁷ Sulpyrine and lysine aspirin and/or tolmetin sodium provocation test responses were positive in all patients, and the patients were in stable condition and had been free of symptoms of respiratory infection for at least 6 weeks (Table I). No subject had ever received pranlukast or other anti-LT drugs that interfere with LT production or responsiveness before this study. All other antiallergic drugs were discontinued for 2 weeks before this study. Other treatments, including inhaled corticosteroids, were all withheld for at least 12 hours before the challenge test, except for slow-release theophylline, which was withheld for at least 24 hours.¹⁸ No patient required regular oral steroid medications. The study was conducted according to the ethical standards of our medical center, which require informed consent from each patient.

Sulpyrine provocation testing

This test was performed with a jet nebulizer driven by compressed air (Nihon Shoji Co, Ltd, Osaka, Japan).

Two tablets of 400 mg acyclovir (total dose of 800 mg) or matching placebo were given orally as pretreatment 80 minutes before the sulpyrine provocation test. A peak acyclovir concentration of 0.94 μ g/mL was reported at 1.31 $\pm$ 0.75 hours after administration of an 800-mg tablet.¹⁹ This is a concentration sufficient to treat herpes virus infection in the phase I clinical study of acyclovir.

Sulpyrine inhibits cyclooxygenase and thus may share a common mechanism of action with aspirin, although its inhibitory activity is less potent than that of aspirin.²⁰ The sulpyrine provocation test is now widely used for diagnosis of aspirin-induced asthma in Japan. Bronchial challenge with sulpyrine was performed according to the standard method of the Japanese Allergy Society as previously described.²¹ Fresh pulverized sulpyrine (Astra Japan, Inc, Osaka, Japan) was dissolved each day in saline to produce reagents.

On the first study day, all patients were given a provocation test after pretreatment with either acyclovir (Nippon Welcome Co, Ltd, Kobe, Japan) or a matching placebo in a double-blind, randomized manner. On the second study day, subjects were crossed over to receive alternative treatment and followed identical the procedure at the same time of day. Each study day began with measurement of FEV₁ and forced vital capacity (FVC) for baseline and then inhalation of nebulized saline to rule out a nonspecific obstructive reaction; FEV₁ was measured 5 minutes before and 5, 10, and 15 minutes after completion of inhalation. If FEV₁ did not fall more than 10%, the subject entered the main part of the study. The sulpyrine provocation test was performed at concentrations as follows: 10, 20, 100, 250, 500, and 1250 mg/mL for all but 1 subject who was too sensitive to challenge at these doses of sulpyrine and therefore 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-induced asthma.

All patients underwent a bronchial provocation test on 2 occasions, separated by at least 2 weeks. Eighty minutes after administration of 2 tablets of 400 mg acyclovir or placebo, inhalation spirometry was performed followed by the provocation test with inhaled sulpyrine. Spirometry was performed again and recorded at 5, 10, 15, and 30 minutes 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 2 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

TABLE II. Bronchial responses in baseline FEV₁ and FVC affected by inhalation of placebo and acyclovir

	Placebo		Acyclovir	
	Before	After	Before	After
FVC	3.49 ± 0.73	3.38 ± 0.80	3.36 ± 0.74	3.40 ± 0.72
FEV ₁	2.03 ± 0.68	2.06 ± 0.63	2.08 ± 0.70	2.07 ± 0.82

constructed and used for calculation of PC₂₀. For statistical evaluation, their PC₂₀ values were set as equal to the highest concentration of sulpyrine given.

Measurement of u-LTE₄

Urine was collected before pretreatment with acyclovir or placebo and at 2- and 4-hour intervals thereafter, and it was stored at -20°C until assay.

LTE₄ was measured by a combined reversed-phase HPLC (Class LC-10 system; Shimadzu, Kyoto, Japan) enzyme immunoassay as previously described.²² 3H-LTE₄ (168.4 Ci/mmol; NEN, DuPont, Dreierich, Germany) was used as the internal standard; antibody to LTE₄ (Cayman Chem, Ann Arbor, Mich) showed cross-reactivity of 10% to 25% versus LTC₄-LTD₄ and less than 0.01% versus LTB₄. Creatinine concentration was measured by using a standard assay kit (Sigma, St Louis, Mo); results, corrected according to the observed recovery, were expressed as the amount of LTE₄ per milligram of creatinine.

Statistical analysis

Statistical evaluation was done with StatView J-4.02 for Macintosh (Abacus Concepts, Inc, Berkeley, Calif). All PC₂₀ values were logarithmically transformed before analysis, and summary statistics were expressed as mean ± SD. Student's paired *t* test was used to compare mean differences. Wilcoxon's signed-rank test was used for comparison of the 2 treatment groups at the same time point regarding FEV₁ and u-LTE₄ values. A *P* value less than or equal to .05 (2-sided) was considered significant.

RESULTS

None of the patients had a severe obstructive reaction to sulpyrine, and all could be followed for longer than 120 minutes. Bronchial responses to acyclovir are presented in Table II. Baseline FEV₁ and FVC were not significantly affected by administration of either acyclovir or placebo. The results showed that we have not confirmed a bronchodilator effect with acyclovir in this study. After pretreatment with acyclovir or placebo, challenge with sulpyrine caused an obstructive reaction in all patients (Table III). After acyclovir, PC₂₀ values were obviously improved in all patients. Furthermore, all PC₂₀ values transformed logarithmically were elevated significantly (Fig 1). Mean log PC₂₀ was 2.12 ± 0.43 (SD) after acyclovir versus 1.18 ± 0.38 before acyclovir (*P* < .0001). The results indicate that oral administration of acyclovir protects against sulpyrine-induced bronchoconstriction almost completely.

Mean baseline u-LTE₄ was 902 ± 242 pg/mg creatinine before acyclovir versus 906 ± 274 pg/mg creatinine on the placebo day (not significant). After placebo, challenge with sulpyrine caused a significant decrease in u-

TABLE III. Individual data for PC₂₀ sulpyrine

Patient no	PC ₂₀ sulpyrine (mg/mL)	
	Placebo	Acyclovir
1	10	100
2	10	100
3	10	250
4	20	100
5	10	100
6	20	100
7	10	100
8	10	1250
9	20	100
10	10	100
11	20	100
12	20	100
13	250	1250
14	10	100
15	20	100
16	4	20

LTE₄, reaching a maximum value of 2304 ± 528 pg/mg creatinine (Fig 2, *P* < .05 compared with baseline). By contrast, after acyclovir, the maximum level of u-LTE₄ was 765 ± 252 pg/mg creatinine, which was significantly lower than placebo (*P* < .05) but not significantly different from baseline. Although the total dose of sulpyrine was higher on the acyclovir day, u-LTE₄ was significantly lower than on the placebo day. No significant correlation was observed between the maximum fall in FEV₁ or the concentration of sulpyrine inhaled and the corresponding maximum increase in u-LTE₄ after placebo or between the protection afforded by acyclovir on FEV₁ decrease and u-LTE₄ excretion.

None of the subjects reported any adverse effect after acyclovir administration, and they could not discriminate between acyclovir and placebo.

DISCUSSION

The mechanism of aspirin-induced asthma remains uncertain. Szczeklik¹⁶ hypothesized that aspirin-induced asthma develops as the result of chronic viral infection. Part of the immunologic response to the infection, expressed by specific cytotoxic lymphocytes, remains under inhibitory control of PGE₂. Removal of PGE₂ by anticyclooxygenase analgesics turns off this control mechanism and allows cytotoxic reactions to proceed. Cytotoxic lymphocytes attack and destroy virus-affected

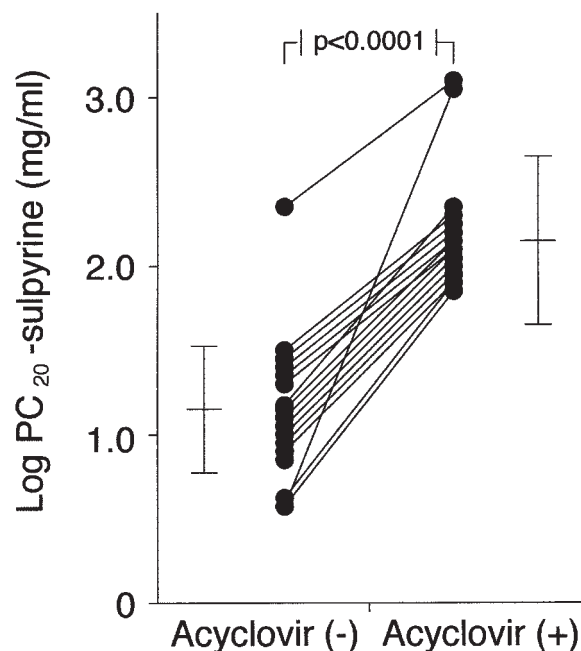


FIG 1. Individual results of PC_{20} sulpyrine at baseline period and after acyclovir. Both results are shown in mean with *bar* indicating SD. All PD_{20} values were logarithmically transformed before analysis, and summary statistics were expressed as mean $\pm$ SD.

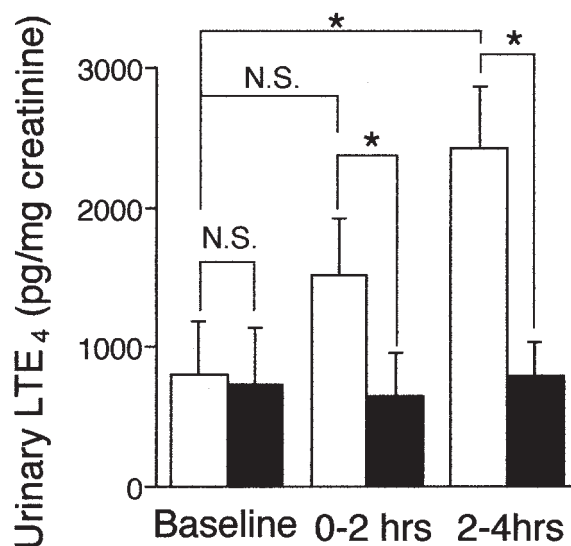


FIG 2. Mean urinary LTE_4 before and during the observation period after placebo (*open bars*) or acyclovir (*filled bars*). * $P < .05$. N.S., not significant.

cells of the respiratory tract. Active oxygen species, toxic metabolites, and mediators released in the course of these reactions precipitate asthma attacks. However, there is no evidence that any virus can cause sporadic cases of aspirin-induced asthma.

It is a widely accepted hypothesis that bronchial overproduction of LTs and inhibition of PG synthesis are

involved in the pathogenesis of aspirin-induced asthma.¹¹⁻¹³ The inhibitory effect of acetylsalicylic acid on the cyclooxygenase increases formation of bronchoconstrictor LTs by means of shunting of unmetabolized arachidonic acid into metabolism by 5-lipoxygenase. The severity and rapidity of bronchospasm induced by cyclooxygenase-inhibiting drugs in aspirin-induced asthma are directly

related to the dose and potency of the drug in inhibiting the cyclooxygenase. Knapp et al²³ showed that excretion of LTE_4 was increased during aspirin-induced asthma episodes, but the degree of increase for individual patients did not correlate with the degree of bronchospasm or inhibition of platelet thromboxane B_2 formation. These results indicate that although the endogenous synthesis of potent bronchoconstrictor LTs increases during aspirin-induced bronchospasm, it is unlikely that a direct shunting of unmetabolized arachidonate into LT synthesis represents the mechanism of aspirin-induced asthma. Our results indicate that pretreatment with acyclovir effectively attenuates analgesic-precipitated bronchoconstriction as previously described.^{2,3}

We demonstrated that oral administration of acyclovir protects against bronchoconstriction of aspirin-induced asthma through mechanisms not related to their bronchodilator properties but rather related to improvement of the hypersensitivity that is evident in almost all patients. These results accord with the findings reported by Nasuhara et al⁴ that acyclovir has no direct effect on the function of airway smooth muscle in the guinea pig. Okada et al² showed acyclovir inhibits both airway obstruction and peribronchial eosinophil accumulation in airway smooth muscle. They conclude that the principal mechanism of the antiasthma effect of acyclovir is inhibition of the release of chemical mediators from mast cells.²

Although it has been thought that acyclovir has no antiallergic or antiinflammatory effects, its chemical structure is similar to that of theophylline.^{2,3} Acyclovir is an acyclic analogue of the natural nucleotide 2-deoxyguanosine.²⁴ On the other hand, theophylline is a derivative of xanthine, a metabolic product of nucleic acids including guanosine.²⁵ Theophylline has been shown to diminish eosinophilia in bronchoalveolar lavage fluid 24 hours after antigen challenge in sensitized guinea pigs²⁶ and 48 hours after inhalation of platelet-activating factor²⁷ and to suppress peribronchial eosinophil infiltration immediately after inhalation of calcium ionophore A23187 .² Moreover, Barnes and Pauwels²⁸ reported that theophylline has antiinflammatory effects in addition to its bronchodilating effects. These findings suggest that theophylline has a suppressive effect on eosinophilia.

The significant drop in u-LTE_4 levels 2 hours after acyclovir administration suggests that acyclovir inhibits LT production or release. Data for preplacebo and postplacebo PC_{20} and preplacebo PC_{20} on the acyclovir day are not significantly different (data not shown), but postacyclovir PC_{20} was higher on the acyclovir day (Table III). These findings confirm that acyclovir is highly effective in preventing aspirin-induced asthma and suggest that this effect is mediated by inhibition of sulfidopeptide LT production or release. The observation that acyclovir also abolishes sulpyrine-induced excretion of u-LTE_4 , a relatively stable metabolite of cysteinyl LTs, strongly suggests that the protective activity is due to inhibition of the release of chemical mediators from bronchial inflammatory cells. There is considerable evidence from pharmacologic studies,²⁹ studies of u-LTE_4 excretion,³⁰⁻³² and studies of

bronchoalveolar lavage fluid³³ that the main inflammatory mediators associated with aspirin-induced bronchoconstriction are cysteinyl LTs that probably originate from eosinophils and mast cells in vivo.³⁴

Although the exact mechanism of acyclovir in bronchoconstriction in aspirin-induced asthma is still unclear, to our knowledge no other article regarding biochemical and physiologic mechanisms has explained such an effect of acyclovir. This is the first report that acyclovir has a protective effect against bronchoconstriction in aspirin-induced asthma, which may be related to its inhibition of LT production or release. However, inhaled sulpyrine is a relatively weak stimulus compared with oral acetylsalicylic acid challenge. The difference between inhalation challenge with sulpyrine and oral challenge with a full therapeutic dose (650 mg) of acetylsalicylic acid should be made clear. To determine the exact relationship between acyclovir and LT production or release in aspirin-induced asthma, additional studies will be required.

In conclusion, we have shown that acyclovir is not only an antiviral drug, but also an inhibitor of bronchoconstriction in aspirin-induced asthma, probably acting by inhibiting production or release of cysteinyl LTs and possibly other chemical mediators produced by bronchial inflammatory cells. This study raises further interesting therapeutic possibilities and warrants further trials of new approaches to the treatment of aspirin-induced asthma.

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REFERENCES

- Schaeffer HJ, Beauchamp L, De Miranda P, Elion GB. 9-(2-Hydroxyethyl)guanine activity against viruses of the herpes group. *Nature* 1978;272:583.
- Okada H, Ohnishi T, Hirashima M, Fujita J, Yamaji Y, Takahara J, et al. Anti-asthma effect of an antiviral drug, acyclovir: a clinical case and experimental study. *Clin Exp Allergy* 1997;27:431-7.
- Taniguchi M, Suetsugu S, Sakakibara H. Anti-viral agent, acyclovir, significantly reduces aspirin-hyperresponsiveness in patients with aspirin-induced asthma. *Am J Respir Crit Care Med* 1997;155:A974.
- Nasuhara Y, Munakata M, Honma Y, Kawakami Y. Acyclovir and guinea-pig airway smooth muscle. *Jpn J Thorac Dis* 1996;33:728-32.
- Harrison WB, Cargeeg A, Bulsara MK, Hanrahan PS. Acyclovir in rheumatoid arthritis. *J Rheumatol* 1993;20:761-3.
- Bagigalupo A, Frassoni F, Van Lint MT. Acyclovir for the treatment of severe aplastic anemia. *N Engl J Med* 1984;310:1606-7.
- Leoni P, Masia C, Offidani M, Da Lio L. Acycloguanosine for the treatment of aplastic anemia. *Eur J Haematol* 1991;46:120-1.
- McDonald JR, Mathison DA, Stevenson DD. Aspirin intolerance in asthma. Detection by oral challenge. *J Allergy Clin Immunol* 1972;50:198-207.
- Delaney JC. The diagnosis of aspirin idiosyncrasy by analgesic challenge. *Clin Allergy* 1976;6:177-81.
- Spector SL, Wangaard CH, Farr RS. Aspirin and concomitant idiosyncrasies in adult asthmatic patients. *J Allergy Clin Immunol* 1979;64:500-6.
- Houjou S, Dobashi K, Nakazawa T. Aspirin-induced asthma. *Jpn J Clin Med* 1994;3:355-8.
- Power I. Aspirin-induced asthma. *Br J Anaesth* 1993;71:619-21.
- Szczeklik A, Schmitz SM. Aspirin-induced asthma: from pathogenesis to therapy. *Allergol Immunopathol* 1993;21:35-40.
- Szczeklik A, Sladak K, Dworski R, Nizankowska E, Soja J, Sheller J. Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1996;154:1608-14.
- O'Sullivan S, Dahlen B, Dahlen SE, Kumlin M. Increased urinary excretion of the prostaglandin D_2 metabolite 9 alpha, 11 beta-prostaglandin F_2

- after aspirin challenge supports mast cell activation in aspirin-induced airway obstruction. *J Allergy Clin Immunol* 1996;98:421-32.
16. Szczeklik A. Aspirin-induced asthma as a viral disease. *Clin Allergy* 1988;18:15-20.
 17. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary diseases (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-44.
 18. Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, et al. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;56:323-7.
 19. Sasa M, Naito Y. Pharmacokinetics of single and multiple doses of oral acyclovir, an antiviral drug. *Jpn J Clin Pharmacol Ther* 1987;18:523-36.
 20. Szczeklik A, Gryglewski RJ, Czerniawska-Mysik G. Relationship of inhibition of prostaglandin biosynthesis by analgesics to asthma attacks in aspirin-sensitive patients. *Br Med J* 1975;1:67-9.
 21. Makino S, Kobayashi S, Miyamoto T, Shida T, Takahashi S. Standard for allergen bronchial provocation tests in asthma and hypersensitivity pneumonitis. *Jpn J Allergy* 1982;31:1074-6.
 22. Sala A, Armetti L, Piva A, Forco GC. An improved assay for urinary LTE_4 . *Prostaglandins* 1994;47:281-92.
 23. Knapp HR, Sladek K, Fitzgerald GA. Increased excretion of leukotriene E_4 during aspirin-induced asthma. *J Lab Clin Med* 1992;119:48-51.
 24. Wagstaff AJ, Faulds D, Goa KL. Acyclovir. A reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* 1994;47:153-205.
 25. Wellman S. Nucleotide metabolism. In: Davidson VL, Sittman DB, editors. *Biochemistry*. 3rd ed. Philadelphia: Harwal Publishing; 1994. p. 489-506.
 26. Sanjar S, Aoki S, Kristersson A, Amith D, Morley J. Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperactivity in sensitized guinea-pigs: the effect of anti-asthma drugs. *Br J Pharmacol* 1990;99:679-86.
 27. Sanjar S, Aoki S, Boubekur K, Chapman ID, Smith D, Kings MA. Eosinophil accumulation in pulmonary airways of guinea-pigs induced by exposure to an aerosol of platelet-activating factor: effect of anti-asthma drugs. *Br J Pharmacol* 1990;99:267-72.
 28. Barnes PJ, Pauwels RA. Theophylline in the management of asthma: Time for reappraisal? *Eur Respir J* 1994;7:579-91.
 29. Nasser SM, Bell GS, Hawksworth RJ, Spruce KE, MacMillan R, Williams AJ, et al. Effect of the 5-lipoxygenase inhibitor ZD2138 on allergen-induced early and late asthmatic responses. *Thorax* 1994;49:743-8.
 30. Christie PE, Tagari P, Ford-Hutchinson AW, Charlesson S, Chee P, Arm JP, et al. Urinary LTE_4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am Rev Respir Dis* 1991;143:1025-9.
 31. Israel E, Fischer AR, Rosenberg MA, Lilly CM, Callery JC, Shapiro J, et al. The pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. *Am Rev Respir Dis* 1993;148:1447-51.
 32. Sladek K, Szczeklik A. Cysteinyl leukotrienes overproduction and mast cell activation in aspirin-provoked bronchospasm in asthma. *Eur Respir J* 1993;6:391-9.
 33. Sladek K, Dworski R, Soja J, Sheller JR, Nizankowska E, Oates JA, et al. Eicosanoids in bronchoalveolar lavage fluid of aspirin-intolerant patients with asthma after aspirin challenge. *Am J Respir Crit Care Med* 1994;149:940-6.
 34. Peter SP, Schulman ES, MacGlashan DW, Scheimen RP, Newball HH, Lichtenstein LM. Pharmacological and biochemical studies of human lung mast cells. *J Allergy Clin Immunol* 1982;69:150.