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Leukotriene (LT)-receptor antagonist is more effective in asthmatic patients with a low baseline ratio of urinary LTE₄ to 2,3-dinor-6-keto-prostaglandin (PG)F_{1α}

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Key words: asthma; leukotriene E₄; leukotriene receptor antagonist; pranlukast; prostaglandin; thromboxane B₂.

Background: To test the hypothesis that urinary levels of arachidonic acid metabolites may be a predicting factor of the effects of pranlukast, a selective leukotriene (LT) antagonist, on chronic adult asthma, we investigated the relationship between its clinical efficacy and urinary eicosanoid levels.

Methods: An open, multicenter trial was conducted involving 38 stable moderate and severe asthmatic patients (mean percent predicted FEV₁ was 71%). All patients received pranlukast (225 mg twice daily) for 4 weeks after a 2-week run-in period. Urinary levels of LTE₄, 11-dehydro-thromboxane (TX) B₂, 2,3-dinor-6-keto-prostaglandin (PG) F_{1α}, and creatinine were measured in 3-h urine collected on day 1 of the treatment. The responder was defined by an improvement of asthma symptom scores and peak expiratory flow rate (PEFR).

Results: One patient was excluded because of an adverse effect, nausea. Thirteen out of 37 subjects were responders and 24 were nonresponders. There were no significant differences in patients' backgrounds and urinary arachidonate levels between the two groups. The urinary LTE₄ to 2,3-dinor-6-keto-PGF_{1α} ratio in the responder was significantly lower ($P=0.01$) than that in the nonresponder. In all patients, a significant inverse correlation was revealed between the baseline urinary LTE₄/2,3-dinor-6-keto-PGF_{1α} ratio and the improvement of PEFR in the morning ($r=-0.43$, $P=0.007$).

Conclusions: These data suggested that the urinary ratio of LTE₄ to 2,3-dinor-6-keto-PGF_{1α} might be one of the predictive markers of the clinical efficacy of this LT-receptor antagonist in asthmatic subjects.

Arachidonic acid released from cell-membrane phospholipid is metabolized to prostaglandins (PGs), thromboxane (TX)

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A₂, and cysteinyl leukotrienes (LTs). LTs, TXA₂, and PGD₂ appear to be potent bronchoconstrictors, and PGE₂ and PGI₂ can protect against airway narrowing (1). LTs exert a thousandfold more potent bronchoconstriction than histamine (2), and increase airway hyperresponsiveness (3), stimulate mucus secretion (4), and augment vascular leakage and mucosal edema (5). These functions are stimulated directly through the LT receptor found on bronchial smooth muscle and other cells in the lung (6). Clinical studies with selective LT-receptor antagonists have been critical in confirming this role (7, 8). It has also been reported that LT generation might be regulated by PGE₂ and PGI₂, and that an increase of LT generation from aspirin-treated leukocytes might be the result of a shortage of endogenous PGE₂, and possibly PGI₂ (9). LTD₄ induces bronchoconstriction directly and indirectly by causing the release of TXA₂ in the guinea pig airway (10). Circulating TXA₂ and PGI₂ are increased after infusion with LTC₄ (9). These eicosanoids may influence each other.

LTE₄ and 11-dehydro-TXB₂ are stable forms of LTs and TXA₂ metabolites, and their urinary levels have been used to reflect the systemic release of their production (11). LTE₄ and 11-dehydro-TXB₂ concentrations in urine are significantly increased in response to allergen challenge in asthmatic subjects (12, 13). PGI₂ is rapidly metabolized to 6-keto-PGF_{1 α} , and enzymatically to 2,3-dinor-6-keto-PGF_{1 α} , which may be an index of systemic PGI₂ biosynthesis (14).

Pranlukast is a competitive LT-receptor antagonist in the guinea pig and man, and was the first one to be used in clinical practice when it was marketed in Japan in mid-1995 (7, 15). Recently, it was reported that pranlukast was well tolerated and effective in chronic asthma treatment in a large population study (16, 17). We hypothesized that the clinical effects of LT-receptor antagonists might differ according to the levels of arachidonic acid metabolites. In this study, we measured urinary LTE₄, 11-dehydro-TXB₂, and 2,3-dinor-6-keto-PGF_{1 α} , and determined whether these metabolite levels predicted the effects of pranlukast on asthmatic patients.

Material and methods

Subjects

The subjects were recruited from Sapporo Medical University Hospital and Tomakomai Prefectural Hospital. A total of 38 subjects (20 women and 18 men, 23–78 years of age) were chosen at random from a larger group of non-

aspirin-sensitive asthmatic patients. All subjects gave informed consent to this study. Asthma was defined by the criteria of the American Thoracic Society (18), and patients were excluded from this study if they had percent predicted FEV₁ over 100%. Patients were required to demonstrate more than 15% improvement of FEV₁ after β_2 -agonist inhalation. The mean resting percent predicted FEV₁ of 38 patients was 71% (range, 38–98%). Patients who had ischemic heart disease, nephritis, or compromised renal or hepatic function, and had moderate or severe asthma exacerbation within 1 month before this trial were excluded from participation. Thirty-four subjects were taking inhaled corticosteroids, 20 subjects were taking inhaled β_2 -agonists, and two subjects had taken 5 mg of prednisolone orally for more than 1 year.

Study design

All subjects received oral pranlukast of 225 mg twice daily for 4 weeks after a 2-week run-in period. At the start of this trial, patients were trained to measure peak expiratory flow rate (PEFR) with the Mini-Wright peak flowmeter (Clement Clark International, Harlow, Essex, UK). They took measurements each morning (between 6 and 8 AM) and evening (between 6 and 8 PM). The best of three performances was recorded as the PEFR.

In the asthma diary, asthma exacerbation, wheezing, cough, sputum, and dyspnea were recorded four times a day (morning, afternoon, evening, and night) from 0 (no symptoms) to 4 (all day), and nighttime sleep was also recorded from 0 (no disturbance of sleep) to 4 (little improvement after inhaler use). We summed up these daily six asthmatic symptom scores (from 0 to 24) for each 2-week period. The clinical effects were assessed every 2 weeks by PEFR in the morning and the asthma symptom scores. The “responder” was defined by both of the following:

- 1) over 15% decrease of symptom scores in the last 2 weeks (3–4 weeks after the pranlukast treatment) from baseline (2 weeks before the treatment)
- 2) over 5% significant increase of PEFR in the 3–4 weeks from baseline.

Three-hour urine for LTE₄, 11-dehydro-TXB₂, 2,3-dinor-6-keto-PGF_{1 α} , and creatinine was collected between 9 and 12 AM on day 1. The urinary eicosanoids were measured after this study was completed.

Table 1. Characteristics of patients, urinary levels of eicosanoids, and response to pranlukast

	Mean $\pm$ SE		P value
	Nonresponder (n=24)	Responder (n=13)	
Sex (F/M)	11/13	8/5	NS
Age (years)	54 $\pm$ 3	59 $\pm$ 3	NS
Duration of asthma (years)	9 $\pm$ 2	11 $\pm$ 3	NS
FEV ₁ , ml	1760 $\pm$ 168	1810 $\pm$ 190	NS
Predicted FEV ₁ , %	69.0 $\pm$ 5.7	72.6 $\pm$ 8	NS
Baseline morning PEFR, 1/min	359 $\pm$ 31	373 $\pm$ 34	NS
Atopy, yes/no	10/14	4/9	NS
Baseline asthma symptom scores	21.6 $\pm$ 1.2	20.8 $\pm$ 1.7	NS
Inhaled steroid, yes/no	22/2	12/1	NS
BDP, μ g/day	916 $\pm$ 105	715 $\pm$ 152	NS
Urinary levels			
LTE ₄ ; pg/mg creatinine	210 $\pm$ 40	168 $\pm$ 34	NS
2,3-dinor-6-keto-PGF _{1α} ; pg/mg creatinine	381 $\pm$ 70	520 $\pm$ 80	NS
11-dehydro-TXB ₂ ; pg/mg creatinine	919 $\pm$ 79	1056 $\pm$ 251	NS
LT E ₄ /2,3-dinor-6-keto-PGF _{1α} ratio	0.78 $\pm$ 0.12	0.32 $\pm$ 0.04	0.01
11-dehydro-TXB ₂ /2,3-dinor-6-keto-PGF _{1α} ratio	4.2 $\pm$ 0.8	2.0 $\pm$ 0.4	NS
11-dehydro-TXB ₂ /LTE ₄ ratio	6.7 $\pm$ 0.9	10.1 $\pm$ 2.0	NS

Baseline; mean daily value of 14 days before this study.
PEFR: peak expiratory flow rate; BDP: beclomethasone dipropionate; LT: leukotriene; TX: thromboxane; PG: prostaglandin.

Measurement of urinary LTE₄

Approximately 30 ml of urine was collected in polypropylene containers from each patient. An internal standard of ³H-labeled LTE₄ (20 000 dpm, 33 pg LTE₄, DuPont New England Nuclear Research, Boston, MA, USA) was added to the remaining sample. Immediately after collection, 4 ml of solution 1 (ethyl acetate:methanol at 2:1) was added to each 1 ml of urine specimen to eliminate proteins; the specimens were then frozen at -70°C until the assays for urinary creatinine and LTE₄ could be performed. All samples were analyzed within 1 month of collection. The method that was used to analyze urinary LTE₄ levels was modified from Tagari et al. (19). Briefly, 5 ml of each urine sample was centrifuged at 2000 g for 15 min to remove any particulates. The supernatant was washed with 3 ml of petroleum ether and centrifuged at 2000 g for 15 min. A volume of 5 ml of 0.05 N acetic acid buffer (pH 4.0) was added to the lower layer to eliminate lipids, loaded into a C8 minicolumn (Bond Elut C8, Varian, Harbor City, CA, USA), and washed consecutively with 4 ml of hexane, 8 ml of solution 2 (hexane:ethyl acetate at 95:5), 4 ml of solution 3 (hexane:ethyl acetate at 30:70), and 4 ml of solution 4 (acetone:

methanol at 50:50). The fraction obtained by solution 4 was evaporated by N₂ gas and reconstructed by 200 ml of solution 5 (methanol:water:acetic acid at 65:35:1, pH 5.6, with triethylamine). A reverse-phase, high-pressure liquid chromatography (RP-HPLC) gradient system equipped with a Nova-Pak C18 column (Waters Assoc., Milford, MA, USA) was used to separate the individual peptido-LT from each other and from other potential non-LT cross-reacting substances, as previously reported by Wescott et al. (20). The sample (150 μ l) was injected into the column at a flow rate of 1 ml/min, and LTE₄ was eluted at 11–13.5 min with this system. Each fraction was evaporated, and the residue was redissolved in 125 ml of the buffer of a commercial radioimmunoassay (RIA) kit; Peptidyl-LT [³H] RIA Kit[®] (DuPont New England Nuclear Research, Boston, MA, USA). LTE₄ was also measured with the same assay buffer kit. The RIA was carried out by the method of Hayes et al. (21) with some modifications. The measurements were corrected by the creatinine content of urine; levels were expressed as picograms per milligram of creatinine. The recovery rate of tritiated LTE₄ was 50–65%. The intra-assay and interassay coefficients of variation were 11% and 10%, respectively.

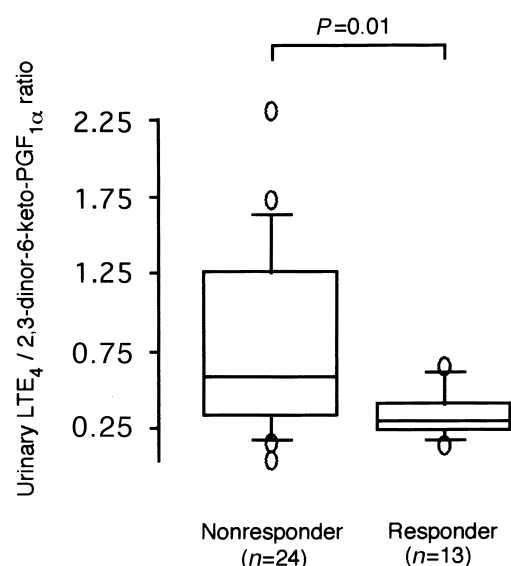


Figure 1. Baseline urinary $\text{LTE}_4/2,3\text{-dinor-6-keto-PGF}_{1\alpha}$ ratio in responders and nonresponders to pranlukast. Rectangles give range from 25th to 75th percentile, horizontal lines indicate median, and vertical lines indicate range from 10th to 90th percentiles.

Measurement of urinary 11-dehydro-TXB₂ and 2,3-dinor-6-keto-PGF_{1α}

Indomethacin was added to the urine sample and stored at -20°C . A volume of 1 ml octadecylsilyl silica (ODS) powder suspension (80 mg ODS/ml 40% ethanol) was added to each 1 ml of urine sample under an acidic condition, to absorb PGs. Then, ethanol-acid solution (15% ethanol with 0.05 N HCl) and petroleum ether were added for washing. Deproteinization and defatting were performed, and PGs were eluted by ethyl acetate. Eluted PGs were applied on a Si minicolumn BOND ELUT SI (Varian, Harbor City, CA, USA) and consequently fractionated by eluent 1 (chloroform:acetic acid at 100:0.5), eluent 2 (acetonitrile:chloroform:acetic acid at 10:90:0.5), and eluent 3 (acetonitrile:chloroform:acetic acid at 25:75:0.5). The fraction obtained by eluent 3 was evaporated by N_2 gas and reconstructed by the buffer of the 6-keto-PGF_{1α} [^{125}I] assay system (Amersham International, Little Chalfont, UK). The value of 2,3-dinor-6-keto-PGF_{1α} was measured with this RIA kit, on the basis of the 31.8% cross-reactivity (22). With the same fraction, 11-dehydro-TXB₂ was measured with the 11-dehydro-TXB₂ [^{125}I] RIA kit (New England Nuclear, Boston, MA, USA). The recovery rates of tritiated 11-dehydro-TXB₂ and 2,3-dinor-6-keto-PGF_{1α} were 61.5% and 67.4%, respectively.

Data analysis

All data are described as mean $\pm$ SE. The differences between the responders and nonresponders were tested with unpaired

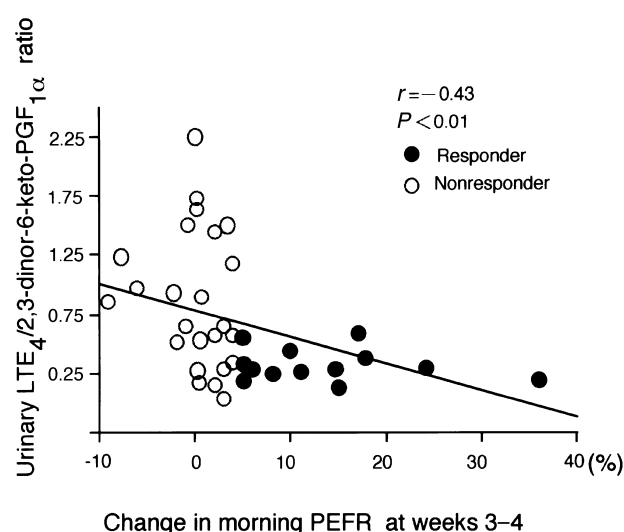


Figure 2. Correlation of baseline urinary $\text{LTE}_4/2,3\text{-dinor-6-keto-PGF}_{1\alpha}$ ratio and mean change of morning PEFR at 3-4 weeks from baseline.

t-tests. The chi-square test was used to test the association of categorical variables. Comparisons of asthma symptom scores, and PEFR between baseline and every 2-week interval were made by analysis of variance (ANOVA) and Scheffe's test. Pearson's least-squares linear-regression analysis was used to determine correlation. Differences with a *P* value of less than 0.05 were considered statistically significant.

Results

Of the 38 patients, one withdrew because of nausea caused by the adverse effect of pranlukast, which disappeared 1 day after the cessation of administration. During this trial, no patient suffered asthma exacerbation; therefore, each regular medication was not changed. Out of 37 patients, 21 had over 15% decrease of asthma symptom scores, and 13 out of 21 had over 5% significant increase of PEFR. Thus, 13 patients were responders and 24 were nonresponders. No significant differences were noted between the two groups in baseline PEFR, percent predicted FEV₁, baseline asthma symptom scores, or any other patient characteristics (Table 1). In the responders, the symptom scores at 3-4 weeks were reduced by 22.2% from baseline (1-2 weeks), but by 5.6% in the nonresponders. Mean changes of PEFR in the responders were significantly ($P < 0.05$) increased in the morning ($7.0\% \pm 2.2$) and evening ($5.9\% \pm 1.8$) in the first 2 weeks, and also significantly ($P < 0.01$) increased in the morning ($13.4\% \pm 2.5$) and evening ($10.9\% \pm 2.2$) at 3-4 weeks. The absolute values of mean increases of PEFR in the responders

at 3–4 weeks were 50 l/min in the morning and 42 l/min in the evening. No nonresponder had over 5 % increase of PEFR in the first 2 weeks and the increases of PEFR at 3–4 weeks were $1.0\% \pm 0.8$ (morning) and $0.3\% \pm 0.8$ (evening).

There were no significant differences in urinary LTE_4 , 11-dehydro-TXB₂, or 2,3-dinor-6-keto-PGF_{1 α} levels between the responders and nonresponders (Table 1). As shown in Fig. 1, only the ratio of LTE_4 to 2,3-dinor-6-keto-PGF_{1 α} of the responders was significantly lower ($P=0.01$) than that of nonresponders. There were no significant differences in the other ratios (Table 1).

All 37 patients revealed inverse correlations between the baseline urinary LTE_4 /2,3-dinor-6-keto-PGF_{1 α} ratio and the improvement of PEFR at 3–4 weeks after pranlukast treatment in the morning ($r=-0.43$, $P=0.007$) (Fig. 2) and the evening ($r=-0.40$, $P=0.01$).

Discussion

This is the first report showing the relation between the clinical efficacy of LT antagonists and the baseline urinary LTs, TX, and PGs levels in asthmatic patients. We found that pranlukast was more effective in patients with a low ratio of urinary LTE_4 to 2,3-dinor-6-keto-PGF_{1 α} , the responders having an imbalance of LTs and PGI₂ generation in terms of relative ratio, although not in absolute levels. A significant inverse correlation between the urinary LTE_4 /2,3-dinor-6-keto-PGF_{1 α} ratio and the improvement of PEFR was also demonstrated. These findings suggested that the baseline urinary ratio of arachidonates might be a predictor of the clinical effects of pranlukast on asthmatic subjects. We need a more direct way to test LT-antagonist treatment on selected patients with a low ratio of urinary baseline excretion of LTE_4 to 2,3-dinor-6-keto-PGF_{1 α} . Recently, we reported that pranlukast treatment over 4 months did not change the urinary LTE_4 /2,3-dinor-6-keto-PGF_{1 α} ratio in asthmatic patients (23).

In our study, the degree of clinical efficacy of pranlukast differed among subjects. Similar results of the effect of pranlukast have been reported (24). Variability in responsiveness to LT antagonists could be explained by differences in the eicosanoid production, the pharmacokinetics of the LT antagonist, the sensitivity to LT receptors, and the involvement of non-LT mechanisms. The interactions among arachidonic acid metabolites might develop individual differences, according to which the production ratios of each eicosanoid would be different. A decreased ratio of LTE_4 to 2,3-dinor-6-keto-PGF_{1 α} might have the potential to

relax bronchial smooth muscle by PGI₂, and a sufficiency of bronchodilating PGI₂ might be effective after the interruption of LT-induced bronchoconstriction by LT antagonists. In this study, we measured the serum levels in 11 subjects (four responders, and seven nonresponders), but there was no significant difference between the two groups (data not shown). Therefore, the absorption of pranlukast may not cause this difference. LTC₄/D₄ receptors are located in the plasma membranes of the smooth-muscle cell in the airway and in other types of cells (25). However, no information on the cloning and expression of the LTC₄/D₄ receptors has been published. The clinical effects of the LT antagonist may be partly affected by the number and sensitivity of LT receptors.

We measured 3-h urine collected between 9 and 12 AM. After intravenous infusion of either synthetic LTC₄ or LTE_4 , 4–7 % was excreted in urine as LTE_4 within 2 h of the infusion (26, 27). Taylor et al. (28) observed an increase of urinary LTE_4 excretion during the first 3 h after an inhaled allergen challenge in allergic asthma, and urinary LTE_4 excretion was significantly higher in subjects with acute severe asthma than in normal controls or stable asthmatic patients. To exclude this influence, we selected the patients without asthma exacerbation within 1 month before this trial. We recently reported circadian rhythmicity of urinary arachidonates. The circadian rhythm of urinary LTE_4 was different among normal, nocturnal, and non-nocturnal asthmatic patients, but there was no circadian rhythm in 2,3-dinor-6-keto-PGF_{1 α} levels (29). The daily mean of these values was close to the level collected between 9 and 12 AM. We used the urine in this period. It seems important to consider circadian rhythmicity in evaluating the urinary arachidonate metabolites.

In our study, the mean value of PEFR at weeks 3–4 was 50 l/min in the responders. This result is consistent with those of a prior study (17); that 4-week double-blind, placebo-controlled, parallel-group study revealed that the pranlukast treatment group significantly increased its PEFR compared to the placebo group (43 l/min vs 15 l/min). On the other hand, Ikeda et al. (24) reported that an observation of PEFR after 2 weeks was enough to evaluate the clinical effects of pranlukast. Our results also revealed that the PEFR of the responders significantly increased in the first 2 weeks, but not that of the nonresponders. A 2-week observation period seemed to be enough to determine the effect of pranlukast.

The limitation of this study was that it was not a double-blind, placebo-controlled study. There remains a slight possibility that placebo responders also had low

baseline urinary LTE₄/2,3-dinor-6-keto-PGF_{1α} ratios. However, this study aimed not to evaluate the efficacy of pranlukast but to determine the difference between the responders and the nonresponders to pranlukast. Our data showed no significant differences in clinical characteristics between the two groups. If the systemic LT/PGI₂ ratio is a predictor of the effects of LT antagonists, urinary measurement may be effective because the procedure is noninvasive.

In conclusion, the present study revealed that the responders to pranlukast had a decreased ratio of urinary LTE₄ to 2,3-dinor-6-keto-PGF_{1α} and suggested that this ratio might be one of the predictive markers of the clinical efficacy of LT-receptor antagonists in chronic asthma patients.

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