Eur Respir J, 1995, 8, 411–415
DOI: 10.1183/09031936.95.08030411
Printed in UK - all rights reserved

European Respiratory Journal
ISSN 0903 - 1936

Prostaglandin D_2-induced bronchoconstriction is mediated only in part by the thromboxane prostanoid receptor

S.L. Johnston*, N.J. Freezer*, W. Ritter**, S. O'Toole*, P.H. Howarth*

Prostaglandin D_2-induced bronchoconstriction is mediated only in part by the thromboxane prostanoid receptor. S.L. Johnston, N.J. Freezer, W. Ritter, S. O'Toole, P.H. Howarth. ©ERS Journals Ltd 1995.

ABSTRACT: Prostaglandin D_2 (PGD_2) is a potent bronchoconstrictor, and is thought to have a role in the pathogenesis of asthma. PGD_2 causes vasodilation acting *via* the prostaglandin (DP) receptor on vascular smooth muscle, and myocontraction acting *via* the thromboxane (TP) receptor on bronchial smooth muscle.

To determine the relative contribution of these mechanisms we have studied the degree to which a potent TP receptor antagonist inhibits PGD_2-induced bronchoconstriction.

Twelve atopic asthmatic subjects underwent baseline PGD_2 bronchial challenges to determine the cumulative concentration of PGD_2 required to reduce forced expiratory volume in one second (FEV_1) by 20%. At four subsequent randomized visits, subjects received this concentration of PGD_2 90 min after dosing with placebo or 20, 50 or 100 mg of BAY u 3405, a potent competitive TP receptor antagonist. Serum was taken for drug assay at 90 min. After each dose of PGD_2, FEV_1 was measured for 30 min, and the area under the percentage fall in the FEV_1/time curve (AUC) was calculated.

The mean±SEM AUC for placebo was 414±68, and for the 20, 50 and 100 mg doses of BAY u 3405 was 169±33, 173±59 and 135±63, respectively. There were no significant differences between the AUCs for any of the drug doses, whilst all three doses were significantly different from placebo. The plateau response achieved with increasing doses of the antagonist suggests complete blockade of the TP receptor.

These data demonstrate that thromboxane receptor blockade only partially inhibits the airway narrowing response to PGD_2, and support the existence of a vascular component to PGD_2-induced acute airway narrowing in asthma.
Eur Respir J., 1995, 8, 411–415.

*Immunopharmacology Group, Southampton General Hospital, Southampton, UK.
**Institute of Clinical Pharmacology, Bayer AG, Wuppertal, Germany.

Correspondence: S.L. Johnston
University Medicine
Level D, Centre Block
Southampton General Hospital
Tremona Road
Southampton
SO9 4XY
UK

Keywords: Asthma
bronchial provocation
prostaglandin D_2
prostaglandin receptor
thromboxane receptor
thromboxane receptor antagonist

Received: May 11 1994
Accepted after revision November 23 1994

There is evidence that the prostanoid mediators prostaglandin D_2 (PGD_2), it's principal metabolite 9α,11β-PGF_2 ($PGF_{2\alpha}$) and thromboxane A_2 (TxA_2) play a role in the pathogenesis of asthma, principally during the early asthmatic response to allergen. PGD_2 is the predominant prostanoid released from human lung mast cells on immunological challenge [1], and produces bronchoconstriction when inhaled by asthmatic subjects at a potency approximately 30 times greater than histamine on a molar basis [2]. Indirect evidence suggests that this mediator class also contributes to airway narrowing provoked by inhaled hypertonic saline [3], and exercise-induced asthma [4]. PGD_2, $PGF_{2\alpha}$ and TxA_2 are all found in increased quantities in the airways of atopic asthmatic subjects following allergen challenge [5–7]; and levels both of PGD_2 and $PGF_{2\alpha}$ are increased in the airways of symptomatic asthmatic compared to both rhinitic and normal subjects [8, 9].

PGD_2 causes vasodilation acting *via* the prostaglandin (DP) receptor on vascular smooth muscle [10], and myocontraction acting *via* the thromboxane (TP) receptor on bronchial smooth muscle [11, 12]. In asthma, the action of PGD_2 has been thought to be mediated principally *via* the TP receptor rather than the DP receptor [12]. As a result, several thromboxane receptor antagonists have now been developed, and have been shown to effectively inhibit PGD_2-induced bronchoconstriction [13–17]. However, these TP receptor antagonists have had less protective effect on allergen-induced bronchoconstriction, with studies reporting only minor and inconsistent effects against the early asthmatic response [13–15].

It is thus possible, that the vascular effects of PGD_2 may be more pertinent than the smooth muscle effects in the acute allergen response. In the upper airways, nasal insufflation with PGD_2 induces nasal blockage both in seasonal and perennial allergic rhinitis [18]. We have recently reported that this obstructive response to PGD_2 within the nose is mediated by the vascular prostanoid (DP) receptor [19]. The acknowledged roles of mucosal swelling and oedema formation in clinical asthma, the disappointing performance of TP receptor antagonists, and the proposed role for the DP receptor in allergic rhinitis suggest that the significance of the vascular effects of prostanoid mediators in asthma may have been underestimated.

To indirectly assess this, we have investigated the maximal airway protective effect of the potent and selective TP receptor antagonist, BAY u 3405, in asthmatic subjects. Previously, we have demonstrated that this antagonist exerts it's maximal effect at a 20 mg dose, with no greater effects being observed with a higher 50 mg dose [16]. We now report the use of increasing doses of BAY u 3405 up to 100 mg, on single concentration challenges with PGD_2, in a placebo-controlled, randomized study to assess the degree to which TP receptor blockade inhibits PGD_2-induced airway narrowing.

Subjects and methods

Subjects

Twelve nonsmoking male asthmatic subjects aged 22–53 yrs (mean age 34 yrs) participated in the study (table 1). All subjects had a baseline forced expiratory volume in one second (FEV_1) of more than 65% of predicted, and a provocative concentration of histamine causing a 20% fall in FEV_1 (PC_{20} histamine) of <1 mg·ml^{-1}. All subjects were atopic, as judged by a wheal >3 mm diameter on skin-prick testing to one or more of *Dermatophagoides pteronyssinus*, house dust, mixed grass pollens, and cat and dog dander (Bencard, Brentford, UK). Their regular treatment consisted of short-acting inhaled β_2-agonists alone, or in combination with an inhaled corticosteroid. Inhaled bronchodilators were withheld for at least 6 h prior to challenge, while inhaled corticosteroids were withheld for a minimum of 12 h. No subject had experienced an exacerbation of asthma within 4 weeks prior to the study. Subjects were asked not to take any aspirin or nonsteroidal anti-inflammatory drugs for 2 weeks prior to and for the duration of the studies. Written informed consent was obtained from each subject and the protocol was approved by the combined Southampton University and Hospitals Ethics Subcommittee.

Bronchial provocation

A screening visit was carried out to determine the cumulative provocative concentration of PGD_2 that produced a 20% fall in FEV_1. This single concentration of PGD_2 was then used for subsequent inhalation challenges.

PGD_2 (Salford Ultrafine Chemicals and Research Ltd, Manchester, UK) was stored at -20°C as a stock solution in pure ethanol at a concentration of 25 mg·ml^{-1}. The identity, purity and concentration of PGD_2 was confirmed by high performance liquid chromatography (HPLC). Solutions were freshly prepared immediately before use by dilution in saline, to produce a range of doubling concentrations from 0.004–4 mg·ml^{-1}.

The solutions were administered at room temperature as aerosols, generated from a starting volume of 2 ml in a disposable Inspiron Mini-nebulizer (CR Bard International, Sunderland, UK) driven by compressed air (8 *l*·min^{-1}). Subjects were instructed to take five consecutive breaths from functional residual capacity to total lung capacity *via* a mouthpiece [20]. Baseline FEV_1 was recorded as the highest of three measurements. Subjects then inhaled five breaths of saline, and FEV_1 was recorded as the higher of two measurements made after 1 and 3 min. Provided the FEV_1 did not fall by ≥10% from the baseline value, provocation with PGD_2 was undertaken. Increasing doubling concentrations of agonist were inhaled at 5 min intervals, and FEV_1 was measured at 1 and 3 min after each inhalation. The challenge was terminated when the FEV_1 fell ≥20% of the higher of the two postsaline values. The percentage fall in FEV_1 from the postsaline value was plotted against the cumulative concentration of agonist on a logarithmic scale, and the cumulative concentration producing a 20% decrease (PC_{20}) derived by linear interpolation. This concentration was then used for each subsequent single concentration challenge, delivered in five breaths as described above.

Table 1. – Characteristics of the asthmatic subjects studied

Subject No.	Age yrs	Sex	Baseline FEV_1 *l*	Baseline FEV_1 % pred	Baseline PC_{20} histamine mg·ml^{-1}	Treatment
1	38	M	3.7	100	0.15	A
2	23	M	3.6	92	0.2	A,B
3	53	M	2.2	69	0.65	A
4	34	M	3.0	87	0.45	A,B
5	44	M	2.7	66	0.9	A,B
6	37	M	2.7	67	0.4	A
7	31	M	4.0	91	0.3	A,B
8	34	M	4.2	102	0.3	A
9	27	M	3.4	86	0.6	A
10	31	M	3.0	77	0.2	A,B
11	22	M	3.8	91	0.04	-
12	33	M	3.9	92	0.15	A
Mean	34		3.4	85	0.275†	
SEM	±2.6		±0.2	±3.5		

M: male; FEV_1: forced expiratory volume in one second; % pred: percentage of predicted; PC_{20}: provocative concentration of

Study protocol

The study was conducted in a placebo-controlled, randomized, double-blind manner. Subjects attended the laboratory on four separate occasions, at the same time of day at least 1 week apart, to undertake bronchial provocation with a single concentration of inhaled PGD_2, calculated to reduce the FEV_1 by at least 20% (see above). Single concentration challenges were carried out 90 min after pretreatment with BAY u 3405 or placebo, as this time-point has previously been shown to be the point at which maximal inhibition of PGD_2-induced bronchoconstriction occurs [16]. Three doses of BAY u 3405 (20, 50 or 100 mg) or matched placebo were randomly administered as three tablets on an empty stomach. Recordings of FEV_1 were made at baseline, and again at 90 min following treatment, when 10 ml of venous blood was withdrawn for assay of the plasma concentration of BAY u 3405. Single concentration bronchial provocation with PGD_2 was carried out, and the FEV_1 recorded immediately prior to challenge, and at 2, 5, 10, 15, 20, 25 and 30 min thereafter. On a separate visit, subjects underwent a saline challenge, and the FEV_1 response was measured over 30 min at the same time-points.

BAY u 3405 assay

The plasma concentrations of BAY u 3405 were determined by gas chromatography, giving a detection limit of 1 $ng \cdot ml^{-1}$. Imprecision was less then 2%, and inaccuracy less than 3% [21]. The assay was performed at Bayer AG, Institute of Clinical Pharmacology.

Statistical analysis

The predrug and postdrug baseline FEV_1 recordings were compared using single factor analysis of variance (ANOVA). For each dose of BAY u 3405 or placebo and for each time-point studied, the PGD_2-induced percentage fall in FEV_1 from the post-treatment baseline was calculated and expressed as the mean±SEM for 12 subjects. The mean±SEM plasma BAY u 3405 concentration was similarly calculated. For each dose of BAY u 3405 or placebo, and for the saline challenge, the total area under the FEV_1 response/time curve (AUC) was calculated by trapezoid integration and expressed as the mean (SEM) for 12 subjects. The percentage falls in FEV_1, for each dose and for placebo were compared using two-way ANOVA. The AUCs were compared between treatment groups and saline using Friedmans two-way nonparametric ANOVA. Comparisons were made between pairs of treatment groups and saline using Wilcoxons test. The null hypothesis was rejected at $p<0.05$.

Results

All 12 subjects completed each of the four study visits, no adverse events were reported. Two of the 12 subjects were unable to attend for the saline challenges, as one had had an exacerbation of asthma and one had left the country. There were no significant differences in baseline FEV_1 between the saline, treatment and placebo days, nor were there any significant differences for any of the three doses used, between baseline FEV_1 and FEV_1 recordings at 90 min after ingestion of BAY u 3405.

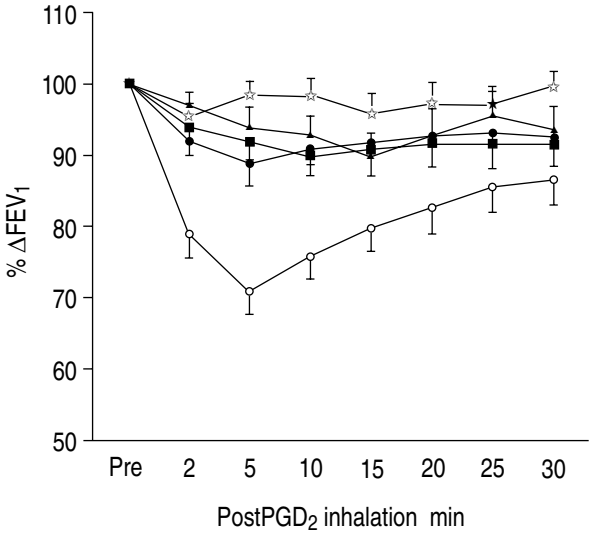

Fig 1. – Percentage fall in FEV_1 (ΔFEV_1) from baseline against time for 30 min following a five breath single concentration inhalation challenge with prostaglandin D_2 (PGD_2), performed 90 min after premedication with placebo or 20, 50 or 100 mg of BAY u 3405 (n=12); and of % ΔFEV_1 against time for the 30 min following a five breath single inhalation challenge with normal saline (n=10). Data are presented as mean±SEM. FEV_1: forced expiratory voume in one second. ○: placebo; ☆: saline; ▲: 100 mg BAY u 3405; ■: 50 mg BAY u 3405; ●: 20 mg BAY u 3405.

On the placebo treatment day, inhaled PGD_2 caused a mean maximal percentage fall in FEV_1, at 5 min after inhalation, of 29.3±3.2% (fig. 1). After the 20, 50 and 100 mg doses of BAY u 3405, the mean maximal percentage falls in FEV_1 were 10.8±3.2, 9.6±2.6 and 9.5±3.4% at 5, 10 and 15 min after inhalation of PGD_2, respectively (fig. 1). The percentage falls in FEV_1 were significantly different from baseline for placebo ($p<0.0001$), and for each dose of BAY u 3405 ($p<0.01$), but there was no significant difference from baseline FEV_1 for the

Table 2. – Magnitude of PGD_2-induced airway narrowing (AUC) after pretreatment with placebo and 20, 50 and 100 mg of the thromboxane receptor antagonist BAY u 3405, and following saline challenge alone

Dosage of BAY u 3405 mg	AUC (n=12) au
0	414±68
20	169±33*
50	173±59*
100	135±63*
Saline	2.8±2.5†

Data are presented as mean±SEM. AUC: area under the % change in FEV_1/time curve; FEV_1: forced expiratory volume in one second; au: arbitrary units. *: $p<0.01$ *cf* placebo; †: p=NS *vs* placebo (n=10).

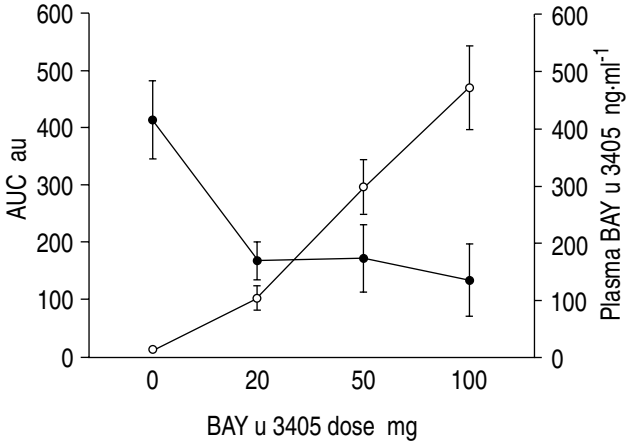

Fig. 2. – Plasma concentrations of BAY u 3405 immediately prior to inhalation challenge with prostaglandin D_2 (—○—), and the response in terms of the area under the percentage change in FEV_1/time curve (AUC) (—●—), for placebo and 20, 50 and 100 mg BAY u 3405. Data are expressed as mean±SEM (n=12).

saline challenge, mean maximal percentage fall in FEV_1 4.6±1.9% (p=NS).

The total responses to the inhaled dose of PGD_2 over the full 30 min postinhalation, expressed as the AUC, were significantly different between each of the three doses of BAY u 3405 and placebo (table 2), the mean AUC for the three doses of BAY u 3405 being 159 arbitrary units (au). There were, however, no significant differences in the AUC between the three doses of BAY u 3405 (fig. 2).

BAY u 3405 was not detected in the plasma of any subject after placebo treatment. The time course of the plasma BAY u 3405 concentrations detected are shown in figure 3. Mean peak plasma concentrations of 110±24, 390±66 and 544±96 ng·ml^{-1} were achieved at 100, 60 and 80 min for the 20, 50 and 100 mg doses, respectively. The estimated mean plasma BAY u 3405 concentrations at 90 min after ingestion, derived by linear interpolation of the 80 and 100 min values, were 103.5±21, 297±47.5 and 471.5±72.5 ng·ml^{-1} for the 20, 50 and 100 mg doses of BAY u 3405, respectively (fig. 2). There was no increase in the protection afforded by the drug against PGD_2-induced airway narrowing with the increasing plasma concentrations obtained after the 20, 50 or 100 mg doses, suggesting that a plateau effect in the magnitude of protection afforded had been achieved (fig. 2).

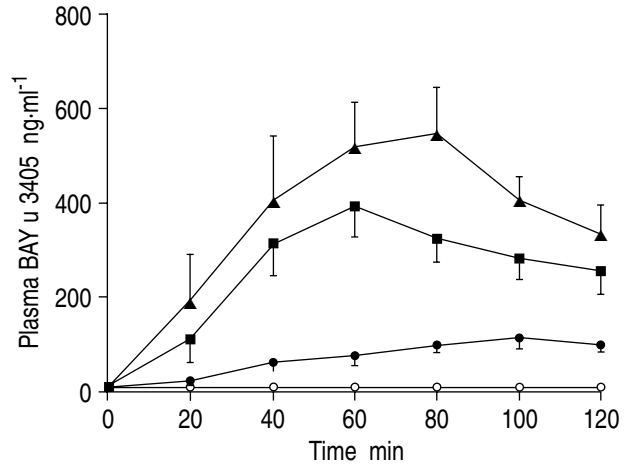

Fig. 3. – Plasma concentrations of BAY u 3405 at various time-points following ingestion of placebo or 20, 50 or 100 mg of BAY u 3405. Inhalation challenge with prostaglandin D_2 was performed at 90 min postingestion. Data are expressed as mean±SEM (n=12). ○: placebo; ▲: 100 mg BAY u 3405; ■: 50 mg BAY u 3405; ●: 20 mg BAY u 3405.

Discussion

This study demonstrates that the TP receptor antagonist, BAY u 3405, produces only partial protection against PGD_2-induced airway narrowing at 90 min after ingestion. The magnitude of protection was similar when 20, 50 or 100 mg doses were used, despite increasing plasma concentrations of BAY u 3405.

As in previous studies, there was no effect of BAY u 3405 on baseline FEV_1 at 90 min after ingestion and prior to agonist challenge, suggesting that TP receptor-mediated bronchoconstriction resulting from increased basal secretion of contractile prostanoids contributes little to baseline airway calibre [12, 16, 17].

The protective effect of BAY u 3405 against the PGD_2-induced airway narrowing observed is considered to be secondary to specific TP receptor antagonism, as BAY u 3405 is known to antagonize all three TP receptor subtypes [19], and previous studies have shown that the drug has no significant effect on the airways response to histamine [16]. *In vitro* studies also confirm no effect against histamine or leukotrienes [22]; therefore there is no evidence of clinically significant functional antagonism.

Previous *in vivo* studies in human asthma confirm *in vitro* and guinea-pig studies indicating that this orally active drug is a potent competitive TP receptor antagonist [16]. BAY u 3405 is one of the most potent TP receptor antagonists for which *in vivo* use has been reported [19], and the degree of protection afforded by BAY u 3405 against PGD_2 challenge is at least as good as that reported for other TP receptor antagonists [16].

The specificity of the TP receptor blockade induced by BAY u 3405 has been shown *in vitro*; in particular, no effect was observed on other prostanoid receptor subtypes, such as DP, EP_1, EP_2, FP or IP [23]. As reported previously for the 20 and 50 mg doses [16], the degree of protection failed to exhibit a dose-response relationship between the 20, 50 and 100 mg doses, a plateau in the magnitude of the protection being observed (fig. 2). In our previous study with BAY u 3405 on PGD_2-induced bronchoconstriction in asthma, a 16 fold shift in the dose response curve to PGD_2 was observed with a median plasma BAY u 3405 concentration of 98.8 ng·ml^{-1} at the time of bronchial challenge [16]. In this study, a similar plasma level was attained at the time of bronchial challenge for the 20 mg dose (103.5±21 ng·ml^{-1}); whilst, as expected, much higher levels were observed for the 50 and 100 mg doses (figs 2 and 3). These levels are well in excess of those needed to achieve effective blockade of TP-receptor-mediated platelet aggregation *in vivo*, around 5–10 ng·ml^{-1} [19].

It is, therefore, probable that both maximal and specific TP receptor antagonism was achieved in this study, and that any residual airway narrowing effect observed after inhalation of PGD_2, will relate to a non-TP receptor effect, mediated through DP receptors, or possibly indirectly *via* cholinergic or lipoxygenase pathways. In this study, the mean AUC calculated for the three doses of BAY u 3405 was 159 au, whilst that calculated for placebo was 414 au, and for the saline challenge was 2.8 au (table 2 and fig 1). These data suggest that, in asthmatic subjects, approximately two thirds of the action of PGD_2 is inhibited by TP receptor blockade on bronchial smooth muscle.

These data may help to explain the disappointing results achieved in models of clinical asthma with TP receptor antagonists [13–15], and, along with the proposed role for the DP receptor in allergen-induced nasal blockage [19], suggest a possible therapeutic role for DP receptor antagonists in the treatment of allergic disease. One such compound has been described [10], but none have yet progressed as far as clinical investigation.

In conclusion, this study has shown that the TP receptor antagonist, BAY u 3405, specifically antagonized the constrictor actions of inhaled PGD_2 when administered orally to patients with asthma. However, the protection afforded was only partial, with a significant proportion of the airway narrowing response not being inhibited. This suggests that the vascular DP receptor may play a more important role in PGD_2-induced lower airway narrowing than has previously been recognized, and raises the potential for a therapeutic benefit of DP receptor antagonism.

***Acknowledgements*:** The authors would like to thank the volunteers who kindly helped with this study, and Bayer AG for providing the study medication.

References

1. Lewis RA, Soter NA, Diamond PT, Austen KF, Oates JA, Roberts LJ II. Prostaglandin D_2 release after activation of rat and human mast cells with anti-IgE. *J Immunol* 1982; 129: 1627–1631.
2. Hardy CC, Robinson C, Tattersfield AE, Holgate ST. The bronchoconstrictor effects of inhaled prostaglandin D_2 in normal and asthmatic men. *N Engl J Med* 1984; 311: 209–313.
3. Finnerty JP, Wilmot C, Holgate ST. Inhibition of hypertonic saline-induced bronchoconstriction by terfenadine and flurbiprofen: evidence for the predominant role of histamine. *Am Rev Respir Dis* 1990; 140: 593–597.
4. Finnerty JP, Holgate ST. Evidence for the roles of histamine and prostaglandins as mediators in exercise-induced asthma: the inhibitory effect of terfenadine and flurbiprofen alone and in combination. *Eur Respir J* 1990; 3: 540–547.
5. Dworski R, FitzGerald GA, Roberts LJ, Oates JA, Schwartz LB, Sheller JR. Eicosanoid formation in atopic human lung: effect of indomethacin. *Am Rev Respir Dis* 1988; 137: 375.
6. Murray JJ, Tonnel AB, Brash AR, *et al.* Release of prostaglandin D_2 into human airways during acute antigen challenge. *N Engl J Med* 1986; 315: 800–804.
7. Wenzel SE, Westcott JY, Smith HR, Larsen GL. Spectrum of prostanoid release after bronchoalveolar allergen challenge in atopic asthmatics and in control groups. *Am Rev Respir Dis* 1989; 139: 450–457.
8. Liu MC, Bleecker ER, Lichtenstein LM, et al. Evidence for elevated levels of histamine, prostaglandin D_2 and other bronchoconstricting prostaglandins in the airways of subjects with mild asthma. *Am Rev Respir Dis* 1990; 142: 126–132.
9. Crea AEG, Nakhosteen JA, Lee TH. Mediator concentrations in bronchoalveolar lavage fluid of patients with mild asymptomatic bronchial asthma. *Eur Respir J* 1992; 5: 190–195.
10. Giles H, Leff P, Bolofo ML, Kelly MG, Robertson AD. The classification of prostaglandin DP-receptors in platelets and vasculature using BW A868C, a novel, selective and potent competitive antagonist. *Br J Pharmacol* 1989; 96: 291–300.
11. McKenniff M, Rodger IW, Norman P, Gardiner PJ. Characterisation of receptors mediating the contractile effects of prostanoids in guinea-pig and human airways. *Eur J Pharmacol* 1988; 153: 149–159.
12. Coleman RA, Sheldrick RLG. Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP-receptors. *Br J Pharmacol* 1989; 96: 688–692.
13. Beasley RCW, Featherstone RL, Church MK, *et al.* The effect of a thromboxane receptor antagonist GR32191 on PGD_2- and allergen-induced bronchoconstriction. *J Appl Physiol* 1989; 66: 1685–1693.
14. Singh V, Wisniewski AFZ, Cooper S, Higgins BG, Tattersfield AE. Effect of ICI 192,605 a thromboxane receptor antagonist on the response to inhaled antigen in asthma. *Am Rev Respir Dis* 1990; 141: A838.
15. Nall K, Dean N, Bleecker E. Effect of a new thromboxane receptor antagonist and bronchial challenge with prostaglandin D_2 and ragweed allergen. *Eur Respir J* 1992; 5: 285s.
16. Johnston SL, Bardin PG, Harrison J, Ritter W, Joubert JR, Holgate ST. The effects of an oral thromboxane TP receptor antagonist BAY u 3405, on prostaglandin D_2- and histamine-induced bronchoconstriction in asthma, and relationship to plasma drug concentrations. *Br J Clin Pharmacol* 1992; 34: 402–408.
17. Fujimura M, Sakamoto S, Saito M, Miyake Y, Matsuda T. Effect of a thromboxane A_2 receptor antagonist (AA-2412) on bronchial hyperresponsiveness to methacholine in subjects with asthma. *J Allergy Clin Immunol* 1991; 87: 23–27.
18. Howarth PH. Allergic rhinitis: a rational choice of treatment. *Respir Med* 1989; 83: 179–88.
19. Johnston SL, Smith S, Harrison J, Ritter W, Howarth PH. The effect of BAY u 3405, a novel thromboxane receptor antagonist, on prostaglandin D_2-induced nasal blockage. *J Allergy Clin Immunol* 1993; 91: 903–909.
20. Chai H, Farr RS, Froelich LA, Mathison DA, McLean JA, Rosenthal RR. Standardisation of bronchial inhalation procedures. *J Allergy Clin Immunol* 1975; 56: 323–327.
21. Ritter W. Determination of BAY u 3405, a novel thromboxane antagonist, in plasma and urine by HPLC and GC. *In*: Reid E, Wilson ID, eds. Bioanalytical Approaches for Drugs, Including Anti-asthmatics and Metabolites. Methodological Surveys in Biochemistry and Analysis, 1992; 22: 211–216.
22. Francis HP, Greenham SJ, Patel UP, Thompson AM, Gardiner PJ. BAY u 3405 an antagonist of thromboxane A_2- and prostaglandin D_2-induced bronchoconstriction in the guinea-pig. *Br J Pharmacol* 1991; 104: 596–602.
23. McKenniff MG, Norman P, Cuthbert NJ, Gardiner PJ. BAY u 3405, a potent and selective thromboxane A_2 receptor antagonist on airway smooth muscle *in vitro*. *Br J Pharmacol* 1991; 104: 585–590.