Prostaglandin D₂-induced bronchoconstriction is mediated only in part by the thromboxane prostanoid receptor


ABSTRACT: Prostaglandin D₂ (PGD₂) is a potent bronchoconstrictor, and is thought to have a role in the pathogenesis of asthma. PGD₂ 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 PGD₂-induced bronchoconstriction.

Twelve atopic asthmatic subjects underwent baseline PGD₂ bronchial challenges to determine the cumulative concentration of PGD₂ required to reduce forced expiratory volume in one second (FEV₁) by 20%. At four subsequent randomized visits, subjects received this concentration of PGD₂, 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 PGD₂, FEV₁ was measured for 30 min, and the area under the percentage fall in the FEV₁/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 PGD₂ and support the existence of a vascular component to PGD₂-induced acute airway narrowing in asthma.


There is evidence that the prostanoid mediators prostaglandin D₂ (PGD₂), its principal metabolite 9α,11β-PGF₂α (PGF₂α) and thromboxane A₂ (TXA₂) play a role in the pathogenesis of asthma, principally during the early asthmatic response to allergen. PGD₂ 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]. PGD₂, PGF₂α and TXA₂ are all found in increased quantities in the airways of atopic asthmatic subjects following allergen challenge [5–7]; and levels both of PGD₂ and PGF₂α are increased in the airways of symptomatic asthmatics compared to both rhinitic and normal subjects [8, 9].

PGD₂ 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 PGD₂ has been thought to be mediated primarily 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 PGD₂-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 PGD₂ may be more pertinent than the smooth muscle effects in the acute allergen response. In the upper airways, nasal insufflation with PGD₂ induces nasal blockage both in seasonal and perennial allergic rhinitis [18]. We have recently reported that this obstructive response to PGD₂ 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 its 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

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age yrs</th>
<th>Sex</th>
<th>Baseline FEV$_1$ l</th>
<th>Baseline FEV$_1$ % pred</th>
<th>Baseline PC$_{20}$ histamine mg·ml$^{-1}$</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>M</td>
<td>3.7</td>
<td>100</td>
<td>0.15</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>M</td>
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<td>0.2</td>
<td>A,B</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>M</td>
<td>2.2</td>
<td>69</td>
<td>0.65</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>M</td>
<td>3.0</td>
<td>87</td>
<td>0.45</td>
<td>A,B</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>M</td>
<td>2.7</td>
<td>66</td>
<td>0.9</td>
<td>A,B</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>M</td>
<td>2.7</td>
<td>67</td>
<td>0.4</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>M</td>
<td>4.0</td>
<td>91</td>
<td>0.3</td>
<td>A,B</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>M</td>
<td>4.2</td>
<td>102</td>
<td>0.3</td>
<td>A</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>M</td>
<td>3.4</td>
<td>86</td>
<td>0.6</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>31</td>
<td>M</td>
<td>3.0</td>
<td>77</td>
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<td>A,B</td>
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<td>11</td>
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<td>-</td>
</tr>
<tr>
<td>12</td>
<td>33</td>
<td>M</td>
<td>3.9</td>
<td>92</td>
<td>0.15</td>
<td>A</td>
</tr>
</tbody>
</table>

Mean SEM

| 34 | 3.4 | 85 | 0.275$^*$ |

M: male; FEV$_1$: forced expiratory volume in one second; % pred: percentage of predicted; PC$_{20}$: provocative concentration of PGD$_2$. 

$^*$ Significant difference from control.
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 PGD₂, calculated to reduce the FEV₁ 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 PGD₂-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 FEV₁ 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 PGD₂ was carried out, and the FEV₁ 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 FEV₁ 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·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 FEV₁ 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 PGD₂-induced percentage fall in FEV₁ 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 FEV₁ response/time curve (AUC) was calculated by trapezoid integration and expressed as the mean (SEM) for 12 subjects. The percentage falls in FEV₁, 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 Wilcoxon's 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 FEV₁ between the saline, treatment and placebo days, nor were there any significant differences for any of the three doses used, between baseline FEV₁ and FEV₁ recordings at 90 min after ingestion of BAY u 3405.

On the placebo treatment day, inhaled PGD₂ caused a mean maximal percentage fall in FEV₁, 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 FEV₁ were 10.8±3.2, 9.6±2.6 and 9.5±3.4% at 5, 10 and 15 min after inhalation of PGD₂, respectively (fig. 1). The percentage falls in FEV₁ 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 FEV₁ for the saline challenge alone.

Table 2. – Magnitude of PGD₂-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

<table>
<thead>
<tr>
<th>Dosage of BAY u 3405 mg</th>
<th>AUC (n=12) au</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>414±68</td>
</tr>
<tr>
<td>20</td>
<td>169±33*</td>
</tr>
<tr>
<td>50</td>
<td>173±59*</td>
</tr>
<tr>
<td>100</td>
<td>135±63*</td>
</tr>
<tr>
<td>Saline</td>
<td>2.8±2.5</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. AUC: area under the % change in FEV₁/time curve; FEV₁: forced expiratory volume in one second; au: arbitrary units. *: p<0.01 cf placebo; †: p=NS vs placebo (n=10).
Inhalation challenge with prostaglandin D2 was performed at 90 min following ingestion of placebo or 20, 50 or 100 mg of BAY u 3405.

**Discussion**

This study demonstrates that the TP receptor antagonist, BAY u 3405, produces only partial protection against PGD2-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 FEV1 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 PGD2-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 PGD2 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, EP1, EP2, 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 PGD2-induced bronchoconstriction in asthma, a 16 fold shift in the dose response curve to PGD2 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 PGD₂, will relate to a non-TP receptor effect, mediated through DP receptors, or possibly indirectly via cholinergic or lipooxygenase 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 PGD₂ 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 PGD₂ 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 PGD₂-induced lower airway narrowing than has previously been recognized, and raises the potential for a therapeutic benefit of DP receptor antagonism.

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**References**


