Br J clin Pharmac 1994; **37**: 97–100

Effects of a thromboxane receptor antagonist on prostaglandin D_2 and histamine induced bronchoconstriction in man

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Many prostanoids including are prostaglandin (PG) $F_{2\alpha}$ and PGD_2 are potent bronchoconstrictor agents. There is evidence to suggest that airway thromboxane (TP) receptor may act as a common receptor for their bronchoconstrictor actions. We tested the hypothesis that inhaled prostaglandin (PG) D_2-induced bronchoconstriction is mediated by interacting with the TP receptor antagonist, ICI 192605, on the bronchoconstrictor response to inhaled PGD_2 in a double-blind, placebo-controlled and crossed-over trial in normal subjects. The effect of ICI 192605 on histamine induced bronchoconstriction served as control for non-specific bronchodilatory actions. The study had two phases; the first consisted of two inhaled PGD_2 challenge study days, and the second phase was that of inhaled histamine. Each study day was separated by at least a week. On each study day, the challenge tests were carried out 30 min after ingestion of 100 mg ICI 192605 or placebo. Doubling concentrations of agonist were given till more than 35% fall in post-diluent specific airway conductance (sGaw) occurred. The concentration needed to cause a fall in a sGaw of 35% post-diluent value (PC_{35}sGaw) was then determined from linear interpolation of the log dose-response. Eight male subjects (median age 26, range 20–35 years) completed the study. ICI 192605 did not change baseline airway calibre 30 min after ingestion on either PGD_2 or histamine study days. ICI 192605 significantly shifted the dose-response curve to inhaled PGD_2 to the right by a median of 3.4 fold (Wilcoxon rank sign test, $P < 0.05$). PC_{35}sGaw PGD_2 (geometric mean with 95% confidence limits) : placebo = 0.49 (0.13–1.85) mg ml^{-1}; ICI 192605 = 1.60 (0.4–6.3) mg ml^{-1}. In contrast, there was no change in PC_{35}sGaw histamine [Placebo = 8.20 (2.95–22.4), ICI 192605 = 6.43 (3.24–12.6) mg ml^{-1}]. The results showed that a TP receptor antagonist inhibited PGD_2, but not histamine, induced bronchoconstriction in man. This supports the hypothesis that inhaled PGD_2-induced bronchoconstriction is mediated by interacting with airway TP receptor.

Keywords thromboxane receptor antagonist PGD_2 and histamine induced bronchoconstriction human

Introduction

The prostanoids have been proposed to be mediators in the pathogenesis of asthma because of their biological activities that are of relevance to asthma [1, 2]. Many prostanoids, such as PGD_2, $PGF_{2\alpha}$ and thromboxane (TX) A_2, are potent contractile agents of airway smooth muscles. There have been considerable debates over the nature of the prostanoid receptors in human airways. It has been suggested that the major bronchoconstrictor prostanoid receptor in human airways is the TXA_2 (TP) receptor, which mediates the bronchoconstrictor actions of other prostanoids as well [3]. If bronchoconstrictor prostanoids exert their actions via the TP receptor in common, then a specific TP receptor antagonist should block their bronchoconstrictor actions. Armour and colleagues have recently shown that a TP receptor antagonist, GR 32191, blocked $PGF_{2\alpha}$, U46619 (a thromboxane mimetic) and PGE_2 induced human bronchial rings contraction by similar degrees, thus providing evidence that all these three prostanoids interacted with airway TP receptors [4]. However, there

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is scanty data to support similar interactions in human airways under *in vivo* conditions. Recently, safe and specific TP receptor antagonists have become available for clinical studies in man. We have investigated whether inhaled PGD_2-induced bronchoconstriction is mediated via airway TP receptors by studying the effects of a TP receptor antagonist, ICI 192605, on inhaled PGD_2-induced bronchoconstriction in normal subjects. To exclude a non-specific effect, the effect of ICI 192605 on histamine induced bronchoconstriction was also studied. The study was randomised, double-blinded with a placebo-controlled and cross-over design.

Methods

Subjects

Healthy, male, non-smoking subjects with normal lung function (FEV_1 > 80% predicted) were recruited into the study. No subject was studied within 6 weeks of an upper respiratory tract viral infection. Subjects were not taking any medications during the study. The study was approved by the ethics committee of the National Heart and Lung Hospital, and written informed consent was obtained from all patients.

Study design

The study was conducted in two phases comprising of PGD_2 (phase 1) and histamine (phase 2) challenges. Each challenge day was separated from each other by at least a week.

Bronchial provocation

Airway calibre was assessed by forced expiratory volume in 1 second (FEV_1) and specific airway conductance (sGaw). FEV_1 was measured with a dry bellow spirometer (Vitalograph, Buckingham, UK), taking the mean of three readings. sGaw was measured with a constant volume body plethysmograph (PK Morgan, UK), and the mean of five readings was used.

On each test day, each subject rested in the laboratory for at least 30 min before commencing the provocation test. After baseline measurement of airway calibre, the subject ingested either ICI 192605 or the placebo according to a predetermined random order, double-blind protocol. Provocation tests were performed 30 min later. Serial dilutions of histamine and PGD_2 solutions were freshly prepared from stock solutions using normal saline as diluent immediately before each bronchial provocation test. All solutions were nebulised from a starting volume of 2 ml by a Wright nebuliser (flow rate 8 l min^{-1}, 0.2 ml min^{-1}) and inhaled by the subject for 2 min with open-mouth tidal breathing using a close fitting mouth-piece. Each subject first inhaled the diluent (normal saline), and if there was no significant bronchoconstriction, doubling concentrations of either PGD_2 or histamine were inhaled till either at least a 35% fall in the post-diluent sGaw or the maximum concentrations of the agonists were reached. The initial provocation concentration of histamine was 1 mg ml^{-1}, with doubling of subsequent concentrations to a maximum of 32 mg ml^{-1}. The starting concentration of PGD_2 was 3.9 μg ml^{-1}, increasing to a maximum of 8 mg ml^{-1}. The fall in sGaw was plotted against the agonist dose on a semi-logarithmic scale, and the provocation concentration of agonist needed to cause a fall in sGaw of 35% (PC_{35}-sGaw) of post-diluent value was determined by linear interpolation. If the final sGaw reached with the maximum concentration was less than 35% of the post-diluent value, then the maximum concentration was taken as the PC_{35}sGaw.

Statistical analysis

Airway calibre data are presented as mean (± s.e. mean) and PC_{35}sGaw data are presented as geometric mean with 95% confidence limits. Paired data were compared with Wilcoxon's rank sum test.

Results

Eight male subjects (median age 26 years, range 20 to 35 years) were recruited and completed the study without any drop-outs. Baseline FEV_1 and sGaw were very similar on all 4 test days (Table 1). There were no significant changes in both FEV_1 and sGaw at 30 min after ingestion of ICI 192605 during both phases of the study. Compared with placebo, ICI 192605 significantly ($P < 0.05$) shifted the dose-response curve to inhaled PGD_2 by median of 3.4 fold to the right. PC_{35}sGaw PGD_2 on placebo treated day was 0.49 (95% confidence limits 0.13 to 1.85) mg ml^{-1}, and on ICI 192605 treated day was 1.60 (0.40 to 6.3) mg ml^{-1}. In contrast, there was no significant change in the dose-response curve to inhaled histamine between ICI 192605 and placebo treated days. PC_{35}sGaw for histamine challenge on placebo treated day was 8.20 (2.95 to 22.4) mg ml^{-1} and on ICI 192605 treated day was 6.43 (3.24 to 12.6) mg ml^{-1} (Table 2). The drug was well tolerated with no side effects.

Table 1 Effect of ICI 192605 on baseline pulmonary function tests. There is no significant difference for both FEV_1 and sGaw between ICI 192605 and placebo treated days for both phases of the study. Values are mean (± s.e. mean)

		PGD_2 challenge days		*Histamine challenge days*	
		Placebo	*ICI 192605*	*Placebo*	*ICI 192605*
FEV_1	Baseline	4.71 (± 2.04)	4.76 (± 0.21)	4.70 (± 0.26)	4.61 (± 0.27)
	Post-drug	4.78 (± 0.25)	4.81 (± 0.23)	4.67 (± 0.26)	4.66 (± 0.26)
sGaw	Baseline	1.26 (± 0.16)	1.22 (± 0.13)	1.46 (± 0.17)	1.29 (± 0.12)
	Post-drug	1.23 (± 0.13)	1.18 (± 0.13)	1.44 (± 0.12)	1.30 (± 0.17)

Table 2 Effect of ICI 192605 on PC_{35}sGaw PGD_2 and histamine

	Placebo	*Active*
PC_{35}sGaw PGD_2 mg ml^{-1} (95% CI)	0.49 (0.1–1.9)	1.60 (0.4–6.3)
PC_{35}sGaw histamine mg ml^{-1} (95% CI)	6.5 (3.0–22)	6.43 (3.2–12.6)

Discussion

Our study has shown that pre-treatment with a TP receptor antagonist, ICI 192605, reduced PGD_2-induced bronchoconstriction. On ICI 192605 treated day, the dose-response curve to inhaled PGD_2 was moved to the right by a median of 3.4 fold as compared with placebo treated day. There was no effect on histamine induced bronchoconstriction. ICI 192605 treatment also did not alter basal airway calibre.

ICI 192605 is a potent, selective and competitive TP receptor antagonist, with pA_2 value of 8.16 for inhibition of human platelet aggregation induced by a thromboxane mimetic, U46619 [5]. With tracheal smooth muscle preparations, ICI 192605 was shown to block contractions induced by U-46619 and not histamine [6]. We are unable to study the effect of ICI 192605 against TXA_2 induced bronchoconstriction *in vivo* because of the short half-life of TXA_2. The thromboxane mimetic, U46619, was also not used because of the risk of causing platelet aggregation. PGD_2 was chosen as the prostanoid to be studied as it is the major prostanoid product of human mast cells [7]. Furthermore, concentration dependent bronchoconstriction can be achieved with increasing inhaled doses of PGD_2 [8]. The inhibition of inhaled PGD_2-induced bronchoconstriction by ICI 192605 suggests that inhaled PGD_2-induced bronchoconstriction is mediated in part by interacting with airway TP receptors. This is in agreement with previous studies that showed that PGD_2-induced airway smooth muscle contraction is mediated through the TP receptor under *in vitro* conditions. In guinea pig trachea, PGD_2 and PGF_{2a} induced contractile responses can be inhibited by a thromboxane receptor antagonist (SQ 29548) [9]. Another thromboxane receptor antagonist, AH23848, can also inhibit PGD_2, PGF_{2a} and U44619 (a thromboxane mimetic) induced contraction of human bronchi [3]. These studies suggest that common TP receptors mediate the contractile responses of other prostanoids under *in vitro* conditions. Our study provides *in vivo* evidence that PGD_2-induced bronchoconstriction is mediated through airway TP receptors in man. Our results are supported by those of Beasley *et al.* [10] showing that another thromboxane receptor antagonist, GR32191, was able to shift the dose-response curve to inhaled PGD_2 to the right in mild asthmatics.

In our study, the attenuating effect of ICI 192605 on PGD_2-induced bronchoconstriction was not due to non-specific functional antagonism as histamine induced bronchoconstriction was not affected. Nevertheless, the prostanoids have been suggested to have a role in bronchial hyperresponsiveness in asthmatics. Inhaled PGD_2 was able to induce hyperresponsiveness to inhaled histamine in man [11]. Treatment with OKY 046, a thromboxane synthetase inhibitor, reduced bronchial hyperresponsiveness to inhaled acetylcholine in asthmatics [12]. The lack of an effect on bronchial responsiveness to histamine by ICI 109625 pre-treatment seen in our study may be due to the different type of subjects that were recruited. The subjects in the OKY 046 study were asthmatics who have increased bronchial responsiveness whereas our subjects were non-asthmatics with normal bronchial responsiveness. In addition, OKY 046 was given for 4 days, as compared with the single dose of ICI 109625 in this study. However, clinical studies with other thromboxane receptor antagonists did not find any significant improvement in allergic asthma [13], exercise asthma [14] and bronchial hyperresponsiveness [15].

In conclusion, a thromboxane antagonist was able to attenuate PGD_2 induced bronchoconstriction without changing histamine induced bronchoconstriction in man *in vivo*. The results support the hypothesis that PGD_2 induced bronchoconstriction is mediated by interacting with airway TP receptors in man.

We would like to acknowledge ICI Pharmaceuticals for providing the material and financial support for the study.

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(Received 1 December 1992, accepted 15 September 1993)