

Effect of GR32191, a potent thromboxane receptor antagonist, on exercise induced bronchoconstriction in asthma

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Abstract

Previous work suggests a role for mast cell derived mediators in exercise induced asthma. The contribution of newly generated contractile prostaglandins to exercise induced asthma was assessed by using a potent and orally active thromboxane (TP₁) receptor antagonist, GR32191. The effect of 120 mg GR32191 on exercise induced asthma was observed in 12 asthmatic subjects. For the exercise challenge the subjects performed six minutes of treadmill exercise, breathing dry air at a work load that had previously been shown to induce a fall in FEV₁ of 25% or more from the pre-exercise baseline. No effect of GR32191 on pre-exercise baseline airway calibre was evident. There was no significant difference in the mean maximum percentage fall in FEV₁ from baseline after exercise between drug and placebo (placebo 30.2%, GR32191 day 31.6%). It is concluded that the thromboxane antagonist GR32191 has no effect on exercise induced asthma. This suggests that prostaglandins, including PGD₂, that act via the thromboxane receptor do not have an important role in exercise induced asthma.

Exercise induced bronchoconstriction in asthma is believed to be due, at least in part, to degranulation of airway mast cells, possibly

as a result of transient hypertonicity of the airway lining fluid.^{1,2} The role of the mast cell has been suggested by studies showing that exercise induced asthma can be suppressed by prior administration of sodium cromoglycate and nedocromil sodium, and partially inhibited by selected H₁ antihistamines.³⁻⁵

Placing hypertonic solutions in the airways has been shown to stimulate the generation and release in vivo of prostaglandin D₂ (PGD₂),⁶ a potent bronchoconstrictor,⁷ in addition to releasing preformed mediators such as histamine. We have shown that the selective cyclo-oxygenase inhibitor flurbiprofen attenuates exercise induced asthma, and this is believed to be through reduction of the endogenous generation of prostanoids, including thromboxane A₂ (TxA₂) and PGD₂.⁵ Previous studies using indomethacin have failed to show any effect on exercise induced asthma.^{8,9} PGD₂ mediated contraction of human bronchial smooth muscle is thought to be mediated via a specific thromboxane TP₁ receptor.¹⁰ The compound GR32191 is a potent orally active competitive TP receptor antagonist, which has been shown to protect the airways of patients with asthma against the bronchoconstrictor effect of inhaled PGD₂ and to reduce the immediate bronchoconstrictor response to inhaled allergen.¹¹ In this study we determined the direct contribution of contractile prostaglandins to the airway narrowing provoked by exercise, using GR32191 in a dose of 120 mg.

Methods

SUBJECTS

Twelve non-smoking asthmatic volunteers (nine male, mean age 28.8, range 19-45 years) took part (table). All were known to have exercise induced asthma. None had had a respiratory tract infection or required any change in medication within a month of entering the study, and none had required oral corticosteroids within the previous six months. All had a baseline FEV₁ of at least 60% of the predicted value on entry to the study, and all gave written informed consent. Before study visits inhaled beta₂ agonists and sodium cromoglycate were withheld for at least six hours and inhaled corticosteroids for at least 12 hours. The study was approved by the Southampton University and hospitals ethical subcommittee.

EXERCISE CHALLENGE

Subjects exercised on an electrically driven

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Subjects' characteristics, baseline values, and maximum responses to exercise challenge (expressed as % fall from pre-exercise FEV₁) after placebo and GR32191

Subject No	Sex	Age (y)	FEV ₁ (% pred)	Daily medication*	FEV ₁			
					Placebo day		GR32191 day	
					l	% fall	l	% fall
1	M	38	113	S, B 200 µg	4.75	45.3	4.55	37.4
2	M	45	89	S	4.00	21.3	3.25	32.3
3	M	32	80	S	3.75	18.7	3.15	39.7
4	F	23	87	D	2.75	29.1	2.70	22.2
5	M	37	65	S	2.15	25.6	2.05	26.8
6	M	19	97	S, B 400 µg	4.50	27.8	4.90	8.2
7	M	39	96	S, B 200 µg	4.00	10.0	3.70	18.9
8	F	21	88	S	2.95	32.2	3.20	21.9
9	M	25	109	S, B 300 µg	4.20	40.5	4.60	28.3
10	M	26	85	T, SC 40 mg	3.75	44.0	3.75	38.7
11	F	19	104	S	4.00	50.0	4.00	45.0
12	M	21	112	S	4.20	17.9	4.20	59.5
Mean (SEM)					3.75 (0.22)	30.2 (3.6)	3.67 (0.24)	31.6 (3.9)

*S—salbutamol; D—combined fenoterol and ipratropium bromide; T—terbutaline (all three taken as required); B—beclomethasone dipropionate; SC—sodium cromoglycate aerosol. All drugs administered by metered dose inhaler.

treadmill (P K Morgan Ltd, Chatham) while inspiring dry air at room temperature and atmospheric pressure from a 200 l Douglas bag via a mouthpiece connected to a two way valve. The volume of inspired air was measured with a Parkinson Cowan gas meter (P K Morgan Ltd) and the volume (BTPS) displayed on the monitor of a microcomputer.

The highest of three measurements of the forced expiratory volume in one second (FEV_1) was taken as the baseline value before exercise testing. Each exercise test lasted six minutes. Air in the Douglas bag was supplemented from an air cylinder as necessary. On completion of the exercise task single measurements of FEV_1 were made at one, three, five, 10, 15, 20, 25, and 30 minutes. The gradient and speed of the treadmill were adjusted during practice tests so that the maximum fall in FEV_1 from the pre-exercise level was 25% or more. The gradient and speed of the treadmill were constant for each subject for each exercise test during the study.

PROTOCOL

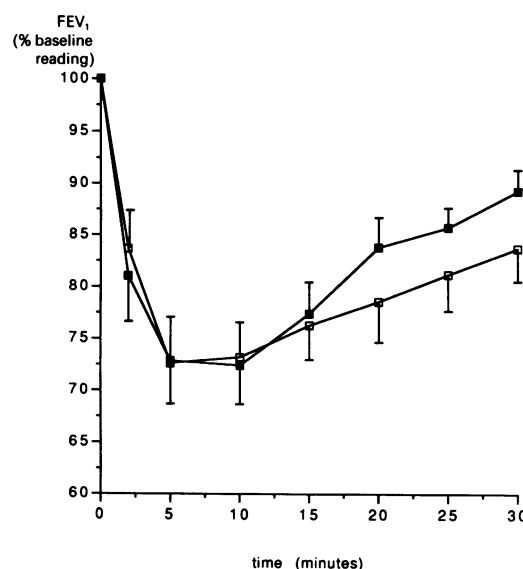
Before entry to the study a treadmill exercise task sufficient to induce at least a 25% fall in FEV_1 from the level immediately before exercise was determined for each subject. The exercise study day visits were at least five and no more than 14 days apart. After the subject had rested for five minutes baseline FEV_1 was measured, the highest of three technically satisfactory readings being used. Either GR32191 120 mg or matched placebo tablets were then administered, the order being randomised. One hour later the pre-exercise baseline FEV_1 was measured, and this was followed by a six minute exercise test. Measurements of FEV_1 were made over the next 30 minutes. Any residual bronchoconstriction was reversed with inhaled salbutamol 200 µg.

DATA ANALYSIS

The maximum percentage fall in FEV_1 from baseline, pre-exercise FEV_1 values, and volume respired during exercise on the two study days were compared by the paired Student's *t* test, significance being attributed at the 5% level. The power of the study to detect a change in the maximum percentage fall in FEV_1 after exercise was calculated from standard statistical tables¹²; for this we used a standard deviation (11.9%) derived from data from the placebo day and from a prestudy control day on the 12 subjects. This figure is consistent with the repeatability of exercise induced bronchoconstriction found by other workers.¹³

Results

The mean (SEM) FEV_1 values before and one hour after GR32191 were 3.69 (0.22) l before GR32191 and 3.67 (0.24) l one hour afterwards (NS). The mean baseline value of FEV_1 immediately before exercise did not differ between the two study days (3.75 (0.22) v 3.67 (0.24) l). There was no significant difference in



Time course of bronchoconstriction induced by exercise, expressed as mean percentage fall from pre-exercise baseline, after GR32191 (closed squares) and placebo (open squares).

ventilation over the six minutes of exercise on the two study days (215 (11) l on the placebo day v 220 (12) l on the GR32191 day).

The maximum percentage fall in FEV_1 from the pre-exercise level after oral placebo was 30.2% (3.6%). Neither the maximum fall in FEV_1 nor the time course of bronchoconstriction differed significantly between placebo and GR32191 (figure and table). Neither a period effect nor a treatment-period interaction was evident. The study had a greater than 80% power of showing a 35% inhibition of the mean maximum percentage fall in FEV_1 .

Discussion

Using an antagonist of thromboxane TP_1 receptors, we have attempted for the first time to separate the component of bronchoconstriction in exercise induced asthma resulting from release of contractile prostaglandins. GR32191 failed to have any significant effect on the magnitude or time course of exercise induced asthma. These data do not support a role for contractile prostaglandins, including mast cell derived PGD_2 , in exercise induced asthma.

Using human bronchial muscle preparations, Coleman and Sheldrick¹⁰ have shown that U-46619, a stable thromboxane mimetic, is the most potent prostanoid contractile agonist, being 383 fold and 628 fold more potent on a molar basis than $PGF_{2\alpha}$ and PGD_2 . As the competitive antagonist AH23848 displaced the dose-response curves for all these prostaglandins to a similar degree they suggested that the prostaglandins mediate their bronchoconstrictor effect via the same receptor, the thromboxane prostaglandin receptor (TP_1).¹⁴ Armour *et al*¹⁵ found that GR32191, an analogue of AH23848, potentially inhibited contractions induced by $PGF_{2\alpha}$ and U-46619 in human bronchial rings, and also protected

against the contractile but not the relaxant effect of PGE₂. Featherstone *et al*¹⁶ found GR32191 to be a potent antagonist in vitro of human airway smooth muscle contraction by PGD₂, PGF_{2α}, PGF_{9α}, PGF_{9α,11β}-PGF₂, and U466191. GR32191 was the most potent of the thromboxane antagonists we studied, and was as potent in protecting against PGD₂ induced contraction as against U466191 induced contraction, with an estimated two fold shift to the right of the dose-response curve at a concentration of the antagonist in the nanomolar range. No effect on methacholine induced contraction was observed at the highest concentration of GR32191 given. The specificity of GR32191 in vitro has been confirmed by other investigators; it does not protect against bronchial muscle contraction induced by carbachol,¹⁵ histamine, 5-hydroxytryptamine, or potassium chloride at concentrations of up to 0.1 mmol.¹⁷ Vasoconstriction by intravenous PGF₂ was ablated by a 20 mg oral dose of GR32191, showing its activity in vivo on vascular contractile TP receptors.¹⁸ PGD₂ induced bronchoconstriction was inhibited after 80 mg of oral GR32191, with a mean 10 fold displacement of the dose-response curve to the right.¹¹ Thus GR32191 is a potent and specific antagonist at the TP₁ receptor in man.

In the present study in patients with asthma, GR32191 failed to increase baseline FEV₁, indicating that contractile prostanoids probably have little influence on resting airway tone. All the subjects studied had an exercise induced fall in FEV₁ and this did not differ significantly in magnitude or time course after GR32191 and placebo. These data suggest that contractile prostanoids have little or no role in the pathogenesis of exercise induced asthma.

This finding accords with the work of O'Byrne and Jones,⁸ who found no inhibition of exercise induced bronchoconstriction with indomethacin. It is, however, in conflict with our previous findings with flurbiprofen, a more potent cyclo-oxygenase inhibitor, which inhibits exercise induced bronchoconstriction, an effect attributed to inhibition of the generation of contractile prostaglandins acting on bronchial muscle.⁵ One possible explanation is that prostaglandins such as prostacyclin, a potent vasodilator generated during hyperventilation,¹⁹ may provoke airway narrowing by vascular engorgement and mucosal swelling. Such an effect would be unopposed by GR32191, which is selective for the TP₁ receptor. A vascular mechanism for exercise induced bronchoconstriction has been suggested by McFadden *et al*,²⁰ who have proposed that airflow limitation in these circumstances is a consequence of rebound hyperaemia, which causes swelling of the bronchial mucosa. Rapid intravenous infusions in asthmatic and normal subjects may induce bronchoconstriction,²¹ presumably by filling bronchial vessels. Four of our subjects were taking inhaled corticosteroids (table), and we might argue, despite the discontinuation of these drugs 12 hours before challenge, that this had inhibited the

contribution of cyclo-oxygenase products to exercise induced asthma in these subjects. Although this is possible for prostanoids from non-mast cell sources, the production of PGD₂ from mast cells is unaffected in vitro by corticosteroids.²²

We have shown that GR32191 fails to inhibit bronchoconstriction induced by exercise in asthmatic subjects. Our findings do not support a role for contractile prostanoids acting through the TP₁ receptor in the genesis of exercise induced bronchoconstriction.

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