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 (TP) 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.

Exercised 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. 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.

Placing hypertonic solutions in the airways has been shown to stimulate the generation and release in vivo of prostaglandin D₂ (PGD₂), a potent bronchoconstrictor, 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 prostanooids, including thromboxane A₂ (TXA₂) and PGD₂. Previous studies using indomethacin have failed to show any effect on exercise induced asthma. PGD₂ mediated contraction of human bronchial smooth muscle is thought to be mediated via a specific thromboxane TP receptor. 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. 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 beta, 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...
treadmill (P K Morgan Ltd, Chatham) while
inspiring dry air at room temperature and
atmospheric pressure from a 2001 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
(FEV₁) was taken as the baseline value before
exercise testing. Each exercise test lasted six
minutes. Air in the Douglas bag was sup-
plemented from an air cylinder as necessary.
On completion of the exercise task single
measurements of FEV₁ 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 FEV₁, 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 exer-
tise task sufficient to induce at least a 25% fall in
FEV₁ from the level immediately before exer-
cise was determined for each subject. The
exercise study day visits were at least five and
no more than 14 days apart. After the sub-
ject had rested for five minutes baseline FEV₁ 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 FEV₁ was measured, and this was
followed by a six minute exercise test.
Measurements of FEV₁ were made over the
next 30 minutes. Any residual bronchocon-
striction was reversed with inhaled salbutamol
200 µg.

DATA ANALYSIS
The maximum percentage fall in FEV₁ from
baseline, pre-exercise FEV₁ 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 FEV₁ 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 pre-study
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) FEV₁ values before and one
hour after GR32191 were 3.69 (0.22) before
GR32191 and 3.67 (0.24) one hour after-
wards (NS). The mean baseline value of FEV₁
immediately before exercise did not differ be-
tween the two study days (3.75 (0.22) v 3.67
(0.24) l). There was no significant difference in
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 FEV₁
from the pre-exercise level after oral placebo
was 30.2% (3.6%). Neither the maximum fall in
FEV₁ nor the time course of bronchocon-
striction differed significantly between placebo
and GR32191 (figure and table 11). Neither a
period effect nor a treatment-period inter-
action was evident. The study had a greater
than 80% power of showing a 35% inhibition
of the mean maximum percentage fall in
FEV₁.

Discussion
Using an antagonist of thromboxane TP₁
receptors, we have attempted for the first time
to separate the component of bronchoconstric-
tion 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 PGD₂, in exercise induced asthma.

Using human bronchial muscle prepara-
tions, Coleman and Sheldrick16 have shown
that U-46619, a stable thromboxane mimetic,
is the most potent prostanooid contractile ago-
nist, being 383 fold and 628 fold more potent on
a molar basis than PGF₂α and PGD₂. As the
competitive antagonist AH23848 displaced the
dose-response curves for all these prosta-
glandins to a similar degree they suggested that
the prostaglandins mediate their bronchocon-
strictror effect via the same receptor, the throm-
boxane prostaglandin receptor (TP₁).14
Armour et al15 found that GR32191, an
analogue of AH23848, potently inhibited con-
tractions induced by PGF₂α in human bronchial
rings, and also protected
against the contractile but not the relaxant effect of PGE₂. Featherstone et al.²⁸ found GR32191 to be a potent antagonist in vitro of human airway smooth muscle contraction by PGD₂, PGF₉α, PGF₁α, PGF₂α, U466191, and U466191. GR32191 was the most potent of the thromboxane antagonists we studied, and was 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,²⁹ histamine, 5-hydroxytryptamine, or potassium chloride at concentrations of up to 0-1 mmol.³¹ Vasorelaxation by intravenous PGF₂, was ablated by a 20 mg oral dose of GR32191, showing its activity in vivo on vascular contractile receptors.³² 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.³³ Thus GR32191 is a potent and specific antagonist at the TP 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,³⁴ 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 prostanoids acting on bronchial muscle.³⁵ One possible explanation is that prostanoids such as prostacyclin, a potent vasodilator generated during hyperventilation,³⁶ may provoke airway narrowing by vascular engorgement and mucosal swelling. Such an effect would be unopposed by GR32191, which is selective for the TP receptor. A vascular mechanism for exercise induced bronchoconstriction has been suggested by McFadden et al.,³⁷ 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,³⁸ 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.³⁹ 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 TP receptor in the genesis of exercise induced bronchoconstriction.