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Effect of RG 12525, an oral leukotriene D_4 antagonist, on the airway response to inhaled leukotriene D_4 in subjects with mild asthma

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We have studied the effect of RG 12525, an oral leukotriene D_4 (LTD_4) antagonist, on LTD_4-induced bronchoconstriction in eight male subjects with mild asthma (baseline $FEV_1 > 80\%$ predicted) in a double-blind, placebo-controlled fashion. RG 12525 800 mg displaced the dose-response curve for LTD_4 to the right. The mean (95% confidence intervals) difference in log $PC_{20}FEV_1$ following RG 12525 and placebo was 2.88 (1.61, 4.17) doubling doses of LTD_4 ($P < 0.01$), a 7.5 fold difference. We conclude that RG 12525 when administered orally is an effective LTD_4 antagonist in subjects with mild asthma.

Keywords RG 12525 leukotriene D_4 antagonist mild asthma

Introduction

Leukotriene D_4 may be an important mediator in asthma since it causes bronchoconstriction in asthmatic and normal subjects when inhaled (Bisgaard *et al.*, 1985; Weiss *et al.*, 1983) and leukotrienes are found in bronchoalveolar lavage fluid (Wardlaw *et al.*, 1989) and urine (Taylor *et al.*, 1989) after antigen challenge in asthmatic subjects. There is interest therefore in leukotriene antagonists as potential treatment for asthma.

Some of the early leukotriene antagonists such as L-649,293, LY-171883 and L-648051 had relatively small effects against inhaled leukotriene D_4 (Barnes *et al.*, 1987; Evans *et al.*, 1988; Phillips *et al.*, 1988) or when given for 6 weeks to asthmatic subjects (Cloud et al., 1989). The newer antagonists, SK&F 104,353-Z_2, ICI-204,219 and MK-571, have shown greater activity against inhaled leukotriene D_4 (Evans *et al.*, 1990; Kips *et al.*, 1990; Smith *et al.*, 1990), allergen challenge (Dahlen *et al.*, 1990) and exercise-induced bronchoconstriction (Manning *et al.*, 1990).

RG 12525, a quinolone derivative, is a competitive antagonist of leukotriene D_4 *in vitro* and protects against inhaled leukotriene D_4 and antigen in sensitized guinea pigs (Carnathan *et al.*, 1989). We have investigated the ability of RG 12525 to antagonise leukotriene D_4-induced bronchoconstriction in subjects with mild asthma.

Methods

Subjects

Eight non-smoking men with mild asthma ($FEV_1 > 80\%$ predicted) aged 18 to 50 years who were otherwise fit were recruited. Entry criteria included treatment with an inhaled β_2-adrenoceptor agonist only, no acute illness or exacerbation of asthma for 6 weeks prior to the study and a repeatable leukotriene D_4 challenge (see below). Each study was carried out at the same time of day with the subject having fasted and abstained from inhaled β_2-adrenoceptor agonists from 24.00 h. Subjects gave written informed consent; the study was approved by the City Hospital Ethics Committee.

Measurements

The forced expiratory volume in one second (FEV_1) was measured with a dry bellows spirometer (Vitalograph®) as the best of three measurements and flow from a partial flow volume curve at 30% above residual volume ($\dot{V}_{30}P$) from a rolling seal spirometer (Ohio®) on line to a microprocessor. The subject wearing a nose clip expired forcefully from end-inspiration after a tidal breath to residual volume. The highest of at least three measurements of $\dot{V}_{30}P$ was used (the two highest within 10%).

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Leukotriene D_4 inhalation challenge Vials of leukotriene D_4 0.1 mg in 0.1 ml absolute alcohol (1 mg ml^{-1}) under argon (Cascade Biochem Ltd, Reading) were stored at −70° C and reconstituted immediately prior to use in sterile phosphate buffered saline (PBS) to provide doubling concentrations from 0.012–50 nmol ml^{-1}. Two ml of each concentration was nebulised by Inspiron nebulizer (Inspiron Bard Ltd), driven by compressed air at 8 l min^{-1} (nebulizer output 0.201 ml min^{-1}; dose leaving nebulizer 0.012 to 50 nmol in 5 min). Subjects inhaled 2 ml PBS for 5 min whilst breathing tidally followed by increasing concentrations of LTD_4 every 15 min until FEV_1 fell by 20% from the post saline value. Blood pressure and heart rate were measured at 5 min and $\dot{V}_{30}P$ and FEV_1 at 10 min post nebulisation.

Protocol

Repeatability study Subjects attended on two occasions at least 2 days apart. After 10 min rest baseline measurements of $\dot{V}_{30}P$ and FEV_1 were followed by a LTD_4 inhalation challenge. Subjects proceeded to the main study only if the provocative concentration of LTD_4 causing a 20% fall in FEV_1 ($PC_{20}FEV_1$) was within two doubling concentrations on the two occasions.

RG 12525 study Subjects attended on 2 further days at least 7 days apart. After 10 min rest heart rate and blood pressure were measured, venous blood taken for haematology and biochemical screen and $\dot{V}_{30}P$ and FEV_1 measured at 5 min intervals until three FEV_1 readings were within 10% of each other (the highest was taken as baseline value). Subjects only proceeded with the second study if baseline FEV_1 was within 10% of that on day 1. The subjects then ingested 800 mg RG 12525 or placebo in a randomized, double-blind fashion. Heart rate, blood pressure, $\dot{V}_{30}P$ and FEV_1 were measured in that order every 30 min for 2 h and venous blood was taken at 2 h for plasma concentration of RG 12525. This was followed by an LTD_4 inhalation challenge starting two doubling concentrations below the PC_{20} value obtained in the repeatability study. The challenge was terminated when the FEV_1 had fallen by 20% or if side effects were troublesome. Inhaled salbutamol (200 μg) was administered if required at the end of the study. Subjects returned 24 to 48 h after each study for a blood test.

Data analysis

FEV_1 and $\dot{V}_{30}P$ were plotted against $\log_{10}$ LTD_4 concentration to obtain the concentration causing a 20% fall in FEV_1 ($PC_{20}FEV_1$) and a 40% fall in $\dot{V}_{30}P$ ($PC_{40}\dot{V}_{30}P$) from the post-saline value by linear interpolation. In four subjects $\dot{V}_{30}P$ fell by 40% after the first dose of LTD_4 following placebo; this dose was assigned as the $PC_{40}\dot{V}_{30}P$. $PC_{20}FEV_1$ and $PC_{40}\dot{V}_{30}P$ values were log transformed prior to analysis. Differences in $PC_{20}FEV_1$ and $PC_{40}\dot{V}_{30}P$ between RG 12525 and placebo days were measured in doubling doses of LTD_4 and geometric mean values compared by paired *t*-test.

Results

Repeatability study

Of the nine subjects recruited one had a difference in $PC_{20}FEV_1$ of more than two doubling doses of LTD_4 and was not studied further (Figure 1). In the remaining eight subjects the mean (s.d.) difference in $PC_{20}FEV_1$ between the two LTD_4 challenges was 0.09 (0.58) doubling doses.

RG 12525 study

Baseline FEV_1 on the two study days did not differ significantly. Over the 2 h following placebo and RG 12525 there was a small increase in mean FEV_1 (4.00 to 4.24 l and 4.01 to 4.18 l respectively) and in mean $\dot{V}_{30}P$ (1.44 to 2.15 and 1.33 to 2.30 l s^{-1}) but no significant difference between the changes following placebo and RG 12525.

Following RG 12525 the dose-response curve for LTD_4 was shifted to the right in all subjects compared with the response after placebo (Figure 2). The geometric mean $PC_{20}FEV_1$ and $PC_{40}\dot{V}_{30}P$ were 0.99 nmol and 0.27 nmol after placebo and 7.34 and 1.96 nmol after RG 12525 (a 7.5 fold change for both). The mean (95% confidence intervals) difference in log $PC_{20}FEV_1$ and log $PC_{40}\dot{V}_{30}P$ following RG 12525 and placebo were 2.88 (1.61, 4.17) and 2.88 (1.22, 4.54) doubling doses of LTD_4 (both $P < 0.01$). There was no correlation between baseline $PC_{20}FEV_1$ and change in $PC_{20}FEV_1$ following RG 12525 ($r = 0.08$).

None of the subjects noticed side effects following RG 12525 or placebo nor any side effects other than a wheeze following inhalation of LTD_4. There was no difference in blood pressure or heart rate after placebo and RG 12525, and no drug related changes in haematological and biochemical screen, urinalysis or electrocardiogram.

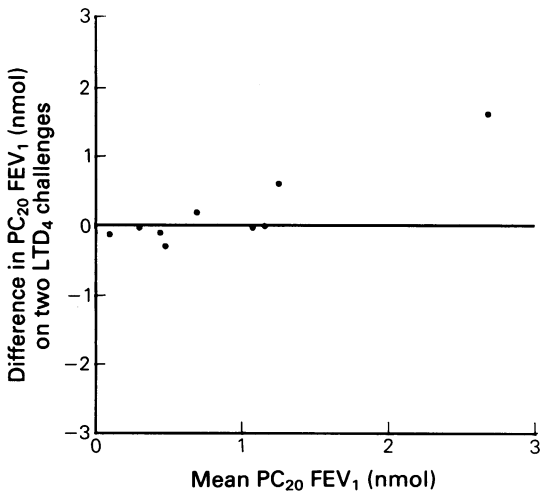

Figure 1 Plot of difference in $PC_{20}FEV_1$ against mean $PC_{20}FEV_1$ from the two LTD_4 challenges to assess repeatability.

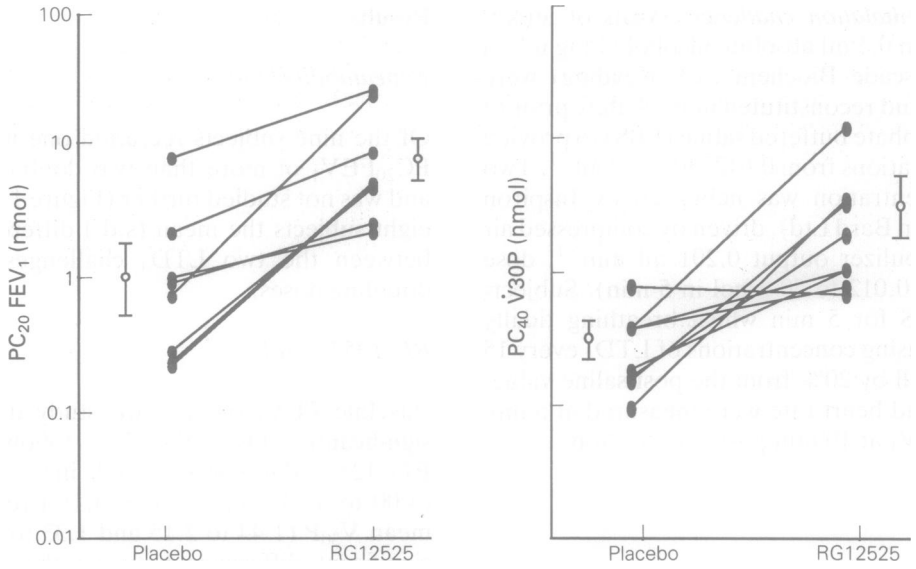

Figure 2 $PC_{20}FEV_1$ and $PC_{40}\dot{V}_{30}P$ following placebo and RG 12525. Open circles represent geometric mean values, bars represent standard error or mean.

Discussion

This study has compared the effect of oral RG 12525 800 mg and placebo on the bronchoconstrictor response to inhaled LTD_4 in eight subjects with mild asthma. There was a small increase in FEV_1 and $\dot{V}_{30}P$ in the 2 h after administration of RG 12525 and placebo, consistent with diurnal variation in lung function. There was no difference however between RG 12525 and placebo and thus no evidence of any bronchodilatation with RG 12525.

RG 12525 caused a 7.5 fold shift of the dose response curve to inhaled LTD_4 (2.88 doubling doses), the mean shift being identical for both FEV_1 and $\dot{V}_{30}P$.

The hypothesis that leukotrienes are important in the pathogenesis of asthma has been supported by some recent studies. A-64077, a 5-lipoxygenase inhibitor, inhibited the bronchoconstrictor response to cold, dry air (Israel *et al.*, 1990), the LTD_4 antagonist ICI-204,219 inhibited the response to allergen challenge in subjects with atopic asthma (Dahlen *et al.*, 1990) and MK-571 inhibited exercise-induced bronchoconstriction (Manning *et al.*, 1990). RG 12525 is a specific and competitive antagonist of LTD_4 in guinea pigs (Carnathan *et al.*, 1989). Our study shows that oral RG 12525 is active in man and has modest activity against LTD_4-induced bronchoconstriction. Since there were no side effects in our study a higher dose of RG 12525 might be tolerated and should produce greater LTD_4 antagonism.

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