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Leukotriene D_4-induced hypoxaemia in asthma is mediated by the cys-leukotriene$_1$ receptor

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ABSTRACT: Bronchoprovocation with cysteinyl-leukotrienes (LTs) induces airflow obstruction and gas exchange abnormalities, namely ventilation-perfusion ratio ($V'A/Q'$) imbalance. However, it is unknown which of the two different receptors for cysteinyl-LTs mediate these $V'A/Q'$ disturbances.

In a double-blinded, crossover design, 10 patients with mild asthma were randomised to receive an oral single dose of the selective cysteinyl-LT$_1$ receptor antagonist montelukast (40 mg) or placebo before leukotriene (LT)D$_4$ inhalation challenge. Gas exchange, including $V'A/Q'$ descriptors were measured at baseline, 3 h after montelukast/placebo pretreatment and 5, 15 and 45 min after the LTD$_4$ challenge.

Compared with montelukast, inhalation of LTD$_4$ induced a marked fall in forced expiratory volume in one second (mean$\pm$SE 33$\pm$2%) and profound $V'A/Q'$ mismatching, reflected by a decreased arterial oxygen tension (from 100$\pm$4 to 75$\pm$3 mmHg) and an increased overall index of $V'A/Q'$ heterogeneity dispersion of retention minus excretion inert gases corrected for dead space (from 4.9$\pm$1.2 to 8.4$\pm$1.1; normal$\leqslant$3.0; dimensionless), 5 min after placebo. Following montelukast, LTD$_4$ produced no significant changes in any of the variables.

In conclusion, these findings point to the view that leukotriene D$_4$-induced gas exchange disturbances and bronchoconstriction are both mediated by the cysteinyl-leukotriene$_1$ receptor.

KEYWORDS: Leukotriene inhalation challenge, leukotriene receptor antagonists, ventilation-perfusion mismatching

It is well established that cysteinyl-leukotrienes (CysLTs), such as leukotriene (LT) C_4, LTD_4 and LTE_4, mediate the major part of airway narrowing induced by allergen in patients with atopic asthma [1]. Likewise, antagonism of CysLTs inhibits significant components of bronchoconstriction evoked by other natural triggers of asthma attacks, such as exercise [2, 3] and exposure to cold-air or irritants [4]. One of the reasons antileukotriene drugs have demonstrated therapeutic efficacy in asthma management [4] is that release of LTs in the airways is a common pathway ultimately shared by many different triggers/inducers of airway obstruction. The ability of CysLTs receptor antagonists (LTRAs) to blunt asthmatic responses to a number of environmental exposures is, according to long-term treatment trials, reflected by less asthma symptoms and fewer exacerbations during the observational period [4]. More recently, reported data suggests that once exacerbations occur, intravenous infusion with montelukast, in addition to standard therapy, causes rapid benefit and is tolerated well in adults with acute severe asthma [5, 6].

Acute asthma attacks clinically present with severe airflow obstruction together with variable gas exchange abnormalities, due to ventilation-perfusion ratio ($V'A/Q'$) imbalance [7]. In line with the role played by CysLTs in airway narrowing, the authors have previously reported that bronchoprovocation with inhaled LTD_4, in addition to intense bronchoconstriction, caused sputum eosinophilia; in particular moderate-to-severe gas exchange disturbances, similar to those occurring in spontaneous acute severe asthma [8]. The observed biological effects of CysLTs are thought to be elicited by the activation of one of two different G-protein coupled receptors, namely $CysLT_1$ and $CysLT_2$ [9]. Constriction of human airway smooth muscle by CysLTs has been mainly associated with activation of the $CysLT_1$ receptor, whereas many

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pulmonary vascular reactions, including contraction and relaxation of human pulmonary blood vessels [10, 11], are initiated at the $CysLT_2$ receptor sites. There have even been findings which suggest the presence of additional CysLT receptors in the human pulmonary vasculature that remain to be characterised at the molecular level [12]. Clinically introduced LTRAs (montelukast, pranlukast and zafirlukast) all block the $CysLT_1$ receptor [4, 9]. As yet there is no $CysLT_2$ receptor antagonist developed for clinical use. In the current study, the term atypical CysLT receptor was used to include $CysLT_2$ receptors as well as other tentative receptors.

The principal objective of the current study was to establish whether or not pulmonary gas exchange disturbances, induced by LTD_4 inhalation in patients with stable-mild asthma [8], were exclusively due to activation of $CysLT_1$ receptors. Therefore, it was hypothesised that part of the V'_A/Q' mismatch could be due to direct pulmonary vascular effects of inhaled LTD_4 on atypical CysLT receptors.

METHODS

Patients

In total, 10 nonsmoking patients (seven females and three males), aged 26±2 yrs, with stable, mild asthma [13] were recruited for the study. The investigation was approved by the Ethical Review Board at Hospital Clínic (Barcelona, Spain) and all patients gave informed written consent. For inclusion, patients were required to have a baseline forced expiratory volume in one second (FEV_1) ⩾1.5 L (⩾70% predicted) and a documented reactivity to inhaled methacholine (MCh; defined as a provocative dose causing a 20% of reduction in FEV_1 (PD_{20}) <1.9 μmol). Patients with an exacerbation of asthma and/or a respiratory infection in the preceding 6 weeks were excluded, as were those treated with oral glucocorticosteroids and antileukotrienes within a 3-months period prior to the study. Maintenance therapy included inhaled glucocorticosteroids (two patients), fixed combined inhaled therapy (three patients), long-acting β_2-adrenergics alone (three patients) and rescue short-acting β_2-adrenergics (two patients). Medication was withheld for >48 h before each visit. Patients were asked to refrain from heavy exercise and consumption of caffeine and tea-containing beverages and foods for >12 h prior to each arrival at the laboratory. No attempt was made to separate atopic from nonatopic patients.

Study design

A randomised, double-blinded, placebo-controlled, two-period crossover study design was used. All patients visited the laboratory on four separate, consecutive occasions. On the first visit (screening), clinical evaluation, spirometry and bronchial challenge with MCh (PD_{20}) were carried out. One week later (second visit), the patients attended the laboratory for a screening rising-dose LTD_4 bronchoprovocation test, that was carried out to assess each patients current sensitivity. One week later, during the double-blinded phase of the study (third and fourth visits), the patients were again challenged with LTD_4 using a fixed two-dose protocol (see below) after administration of the LTRA montelukast (four 10 mg tablets) or placebo (lactose), one week apart. All sets of measurements were performed each day at baseline (B0), 3 h after montelukast or placebo administration (B1), and then after LTD_4 challenge, at 5 (nadir), 15 and 45 min. Although previous studies have shown no significant difference in antagonism of LTD_4 bronchoconstriction between 5, 20 and 100 mg of montelukast [14], a dose of 40 mg (approximately half a log higher than the standard clinical dose of 10 mg) was selected for this study in order to make up for possible individual variability in oral absorption and to guarantee effective antagonism in all individuals. This, slightly higher dose had no effect on atypical CysLT receptors in preclinical models [9, 15], and much higher doses (250 mg) have shown no adverse effects [14]. At the end of each study day the patients received 300 μg of inhaled salbutamol and were not allowed to leave until FEV_1 had returned to ±5% of the prechallenge baseline values. All study days were completed by all the patients without any side effect, except for those locally related to the arterial and venous puncture sites.

LTD_4 challenge

The rising dose bronchoprovocation with LTD_4 (long-protocol) performed during the second visit had been validated [16] and used previously [8]. In brief, the protocol results in the inhalation of three-fold increasing cumulative doses of LTD_4 (Good Manufacturing Practice; Cascade Biochemicals, Reading, UK; supplied in an ethanol and water ratio of 1:2–1:4), from a dosimeter-controlled jet nebuliser (Spira Elektro 2; Respiratory Care Center, Hameenlinna, Finland) by varying the number of breaths between one, two and seven and by using colour-coded vials of LTD_4 with 10-fold increasing concentrations. Challenges always began with the inhalation of the vehicle. The dosimeter was set to nebulise 8 μL of solution per breath during 0.6 s, starting after the inhalation of 100 mL of the tidal volume with an inspiratory flow rate of 0.5 $L \cdot s^{-1}$. After each set of inhalation, FEV_1 was measured at 5 and 10 min and the inhaled dose was increased until there was >25% fall from baseline. Accordingly, both LTD_4-PD_{20} and LTD_4-PD_{25} were calculated from the relationship between the cumulated dose and the airway response. LTD_4-PD_{20} was analysed as a standard only approach for responsiveness, while the greater dose (LTD_4-PD_{25}) was used to guarantee a pronounced and consistent response during the next double-blind phase of the trial. In order to standardise the LTD_4 bronchial challenges during the treatment period, the patients inhaled the same total dose of LTD_4 at visits three and four, corresponding to their individually established PD_{25} at screening, LTD_4 was administered as two consecutive dose steps on each day (named short-protocol). Thus, one-third of the LTD_4 total dose was given followed by the remaining two-thirds, 10 min apart, ensuring the same three-fold increase in inhaled LTD_4 dose in total. FEV_1 was measured at 5 and 10 min after each of these two steps. During each LTD_4 challenge, the patients were seated and breathed room air.

Outcome measures

After ensuring the establishment of adequate steady-state conditions, demonstrated by stability of ventilatory and haemodynamic variables and by the close agreement between mixed expired O_2 and CO_2 (±5%), a set of duplicate measurements was performed in the following sequence: respiratory system resistance (Rrs); FEV_1; arterial blood and mixed expiratory inert and respiratory gases samplings; and ventilatory and haemodynamic measurements. The forced

oscillation technique was used to measure *R*rs, its analysis was restricted to 5 Hz [17]. A three-lead electrocardiogram, heart rate, and systemic arterial pressure and arterial oxygen saturation through a pulse oxymeter (HP M1166A; Hewlett-Packard, Bollinger, Germany) were continuously recorded throughout the study (HP 7830A Monitor and HP 7754B Recorder; Hewlett-Packard, Waltham, MA, USA). Blood samples were collected anaerobically through a catheter inserted into the radial artery. Arterial partial pressure of oxygen, carbon dioxide tension, pH and haemoglobin concentrations were analysed using standard electrodes (800 series; Ciba Corning, Medfield, USA). Oxygen uptake (V'_{O_2}) and carbon dioxide production (V'_{CO_2}) were calculated from mixed expired O_2 and CO_2 concentrations respectively, measured by infrared cell (MedGraphics, St Paul, MN, USA). Minute ventilation and respiratory rate were measured using a calibrated Wright spirometer (Respirometer MK8; BOC-Medical, Essex, UK). The alveololar-arterial oxygen tension difference (P_{Aa,O_2}) was calculated according to the alveolar gas equation using the measured respiratory exchange ratio. The multiple inert gas elimination technique was also used to estimate the distributions of V'_A/Q' ratios without sampling mixed venous inert gases in the customary manner, a modality that has shown similar accuracy [18]. With this approach cardiac output is directly measured by dye dilution technique (DC-410; Waters Instruments Inc, Rochester, MN, USA) using 5 mg bolus of indocyanine green injected through a central catheter placed percutaneously by an arm vein, while mixed venous inert gas concentrations are computed from mass balance equations [18]. The principal variables assessed were the dispersions of pulmonary blood flow (Log SDQ normal values $\leqslant$0.60) and of alveolar ventilation (Log SDV; normal values $\leqslant$0.65) [19] and an overall index of V'_A/Q' heterogeneity dispersion of retention minus excretion inert gases corrected for dead space (DISP R-E*), namely the root mean square difference among measured retentions (R) and excretions (E) of the inert gases (except acetone) corrected for the dead space (normal value$\leqslant$3.0; all dimensionless) [20]. Intrapulmonary shunt and low V'_A/Q' mode were defined as the fraction of blood flow perfusing lung units with V'_A/Q' ratios <0.005 and <0.1 (excluding shunt), respectively. Dead space and high V'_A/Q' mode were defined as the fraction of alveolar ventilation to lung units with V'_A/Q' ratios >100 and >0.1 (excluding dead space), respectively. The duplicate samples of each set of measurements were treated separately, with the final data resulting in the average of variables determined from both V'_A/Q' distributions at each time point. In one patient, inert gas data samples were not available at 45 min one study day due to technical difficulties.

Statistical analysis

All data are expressed as mean$\pm$SE or 95% confidence intervals. PD20 and PD25 values for MCh and LTD_4 were derived by linear interpolation from the respective log dose-cumulated dose-response curves. Their geometric means were calculated on log-transformed raw data. The effects of montelukast or placebo pretreatment before LTD_4 challenge were assessed using paired t-test. The effects of LTD_4 challenge and the interaction between montelukast or placebo pretreatment were assessed using two-way repeated measures ANOVA. Whenever a significant interplay between the effects of LTD_4 challenge and those shown after intervention were observed, differences between montelukast and placebo for each time point (at 5, 15 and 45 min after LTD_4) were analysed with a *post hoc* paired t-test. Pearson's correlation test was used when necessary. Statistical significance was set at $p<0.05$ values in all instances.

RESULTS

Baseline findings

Patients had normal FEV1 (3.2$\pm$0.2 L; 88$\pm$3% pred) and gas exchange values, with mild increases in *R*rs alone. As expected, distributions of pulmonary blood flow and alveolar ventilation were narrowly unimodal, while intrapulmonary shunt and areas with low and high V'_A/Q' ratios were conspicuously absent. Dead space was within expected limits. Except for FEV1, which decreased in the placebo arm (from 3.3$\pm$0.3–3.1$\pm$0.3 L; $p<0.05$), no other significant differences in any of the other outcome measures were shown between measurements carried out before and after montelukast or placebo pretreatment (table 1).

LTD_4 challenge

At the second visit, the long-protocol of LTD_4 challenge produced a significant bronchoconstriction, as shown by a severe decrease in FEV1 (by 32$\pm$2%; range 20–47%) in all of the patients. The corresponding geometric mean PD20 values were 0.77 nmol (range 0.13–3.26 nmol), and 388.7 nmol (range 150–1,020 nmol), for LTD_4, and MCh, respectively. The mean difference in molar potency of LTD_4 and MCh was ~400. A LTD_4 challenge, during both long- and short-protocols after placebo pre-treatment produced a similar FEV1 reduction (by 32$\pm$2 and 33$\pm$2%, respectively). The corresponding geometric mean LTD_4-PD25 values (n=7) were 1.06 nmol (range 0.025–6.74 nmol) during the long-protocol and 0.57 nmol (range

TABLE 1 Baseline function data on placebo and montelukast studies

	Placebo		Montelukast	
	B0	B1	B0	B1
FEV1 % pred	89$\pm$5	84$\pm$6*	84$\pm$4	82$\pm$4
***R*rs cm $H_2O\cdot L^{-1}\cdot s^{-1}$**	4.4$\pm$0.3	4.8$\pm$0.4	4.5$\pm$0.4	4.6$\pm$0.4
P_{a,O_2} mmHg	102$\pm$3	100$\pm$4	102$\pm$4	103$\pm$3
P_{a,CO_2} mmHg	39$\pm$2	38$\pm$2	41$\pm$2	39$\pm$2
P_{Aa,O_2} mmHg	2$\pm$1	5$\pm$2	6$\pm$4	4$\pm$2
Log SDQ	0.50$\pm$0.04	0.57$\pm$0.06	0.56$\pm$0.07	0.56$\pm$0.05
Log SDV	0.51$\pm$0.05	0.58$\pm$0.07	0.49$\pm$0.05	0.55$\pm$0.05
DISP R-E*	3.5$\pm$0.5	4.7$\pm$1.1	3.7$\pm$0.7	4.2$\pm$0.7

Data are presented as mean$\pm$SE. B0: Baseline; B1: 3 h after baseline; FEV1 % pred: forced expiratory volume in one second percentage predicted; *R*rs: total resistance of respiratory system; P_{a,O_2}: arterial oxygen tension; P_{a,CO_2}: carbon dioxide arterial tension; P_{Aa,O_2}: alveolar-arterial oxygen tension difference; Log SDQ: dispersion of blood flow distribution (dimensionless); Log SDV: dispersion of alveolar ventilation (dimensionless); DISP R-E*: dispersion of retention minus excretion inert gases corrected for dead space (dimensionless). 1 mmHg=0.133 kPa. *: $p<0.05$ compared with B0.

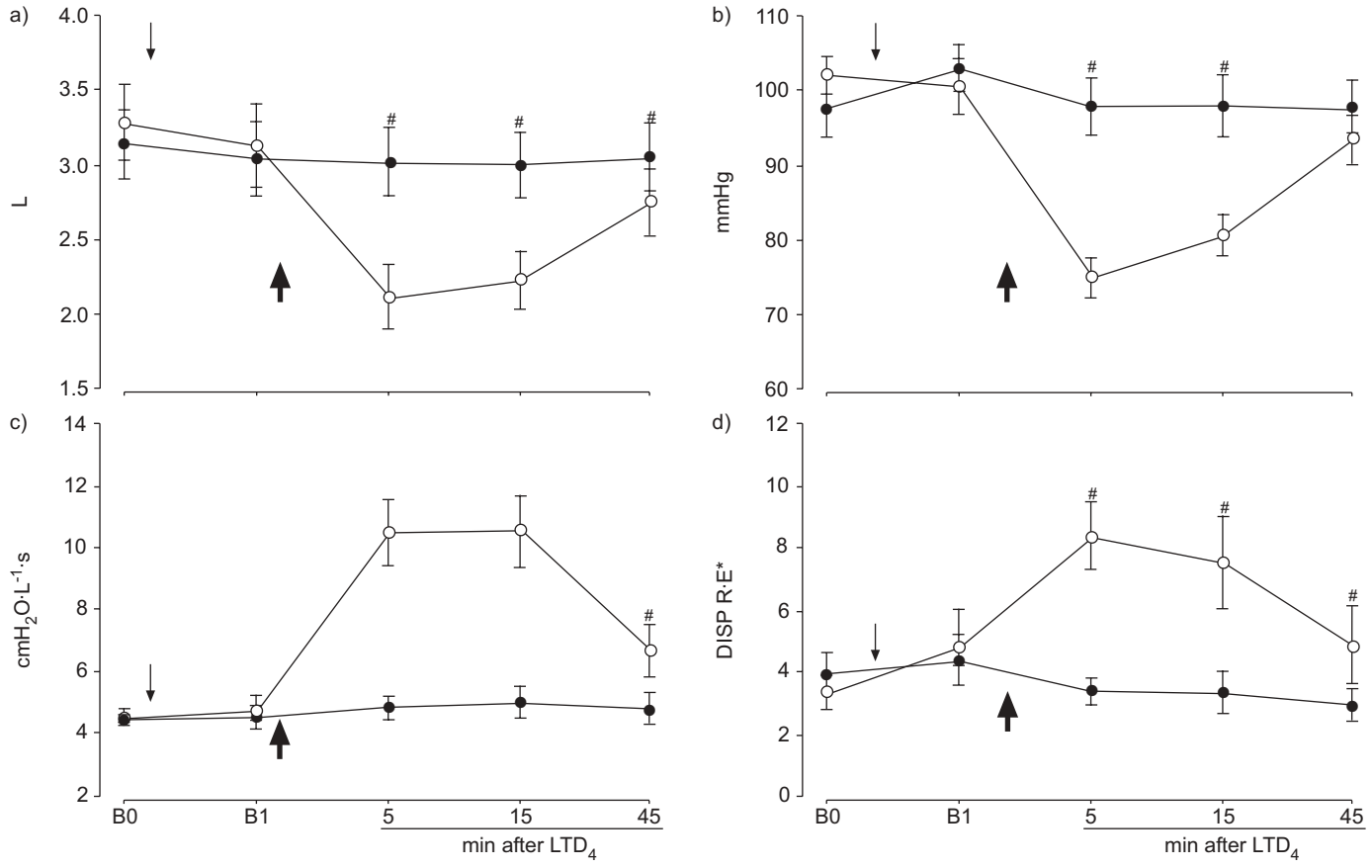

FIGURE 1. Time courses (mean±SEM) for a) forced expiratory volume in one second, b) arterial oxygen tension, c) respiratory system resistance and d) ventilation perfusion mismatch (expressed as DISP R-E*; dimensionless). These were measured at baseline (B0), 3 h after baseline (B1) and at 5, 15 and 45 min after leukotriene D_4 (LD_4). ●: pretreatment with montelukast; ○: pretreatment with placebo. Thin arrows represent montelukast or placebo. Bold arrows represent LD_4 challenge. #: denote significant differences between placebo and montelukast at each time point. See Results section for p-values.

0.021–2.21 nmol) during the short-protocol after placebo ($p=0.40$). LTD_4-PD_{25} values could not be calculated in three patients because FEV1 already fell >25% at the first step during the short-protocol.

LTD_4 effects after placebo

Challenge with LTD_4 produced, as compared with montelukast, a significant bronchoconstriction as shown by a marked FEV1 decrease (33±2%; $p<0.0001$) and a considerable increment in *R*rs (124±15%; $p<0.001$; table 2; fig. 1). Although with less severity, the latter two parameters were still significantly different at 15 and 45 min. This intense bronchoconstriction was associated with mild-to-moderate disturbances in pulmonary gas exchange in most of the patients (table 2; fig. 1). This was essentially characterised by decreases in arterial oxygen tension (P_{a,O_2}; 26±4 mmHg; $p<0.001$) and increases in P_{Aa,O_2} ($p<0.0001$), Log SDQ ($p<0.01$), Log SDV ($p<0.05$) and DISP R-E* ($p<0.01$). Of note, three patients had further deteriorated P_{a,O_2} and V'_A/Q' indices at 15 min, a point in time at which most of the pulmonary gas exchange variables were still abnormal; at 45 min only DISP R-E* ($p<0.05$) values remained marginally increased. All but two patients showed mild-to-moderate hypoxaemia (<80 mmHg) either at 5 or 15 min after the LTD_4 challenge. All V'_A/Q' distributions at the nadir of challenge and/or at 15 min were broadened without areas of low or high V'_A/Q' ratios, while both intrapulmonary shunt and dead space remained unchanged. All the other ventilatory and haemodynamic variables and gas exchange indices, including V'_{O_2} and V'_{CO_2}, remained unchanged after inhalation of LTD_4 throughout the whole of the study period.

Patients baseline FEV1 ($r=-0.72$; $p<0.05$), P_{a,O_2} ($r=-0.77$; $p<0.01$) and P_{Aa,O_2} ($r=0.65$; $p<0.05$) were closely correlated with their respective differences after LTD_4 challenge. Differences in Log SDV and in DISP R-E* before and immediately after LTD_4 bronchoprovocation were also closely correlated ($r=0.66$; $p=0.05$; and $r=0.70$; $p<0.05$) with their corresponding baseline values, respectively. By contrast, no correlations between spirometric or lung mechanic parameters and pulmonary gas exchange descriptors were shown.

LTD_4 effects after montelukast

As compared with placebo, pretreatment with montelukast completely blocked in all but one patient (patient no. 5; fall in FEV1 16%) the bronchoconstriction induced by LTD_4: both FEV1 and *R*rs remained essentially unvaried (1±2% and 8±7%, respectively; table 2; fig. 1). Likewise, pulmonary gas exchange values remained stable and only one patient

TABLE 2 Changes induced by leukotriene D_4 challenge after placebo (P) or montelukast (M) pretreatments

	5 min	15 min	45 min	p-values
FEV_1 % pred				<0.001
P	-33 (-38– -28)#	-28 (-35– -22)#	-11 (-20– -1)***	
M	0 (-5–5)	-1 (-5–3)	1 (-3–4)	
***R*rs cm $H_2O \cdot L^{-1} \cdot s$**				<0.01
P	5.7 (3.8–7.6)#	5.8 (3.6–8.0)***	1.9 (0.4–3.4)*	
M	0.3 (-0.4–0.9)	0.5 (-0.2–1.1)	0.2 (-0.4–0.8)	
***P*$_{a,O_2}$ mmHg**				<0.01
P	-26 (-35– -16)***	-20 (-28– -12)**	-7 (-13– -1)	
M	-5 (-12–2)	-5 (-12–2)	-5 (-11–0)	
***P*$_{a,CO_2}$ mmHg**				NS
P	1 (-1–2)	1 (-1–3)	0 (-2–2)	
M	3 (1–6)	3 (1–5)	1 (0–2)	
***P*$_{Aa,O_2}$ mmHg**				<0.01
P	21 (16–26)#	17 (10–24)*	5 (0–10)	
M	3 (-1–7)	3 (-1–9)	3 (-2–7)	
Log SDQ				<0.05
P	0.28 (0.13–0.44)**	0.19 (0.09–0.29)*	0.01 (-0.08–0.09)	
M	-0.02 (-0.14–0.10)	-0.02 (-0.17–0.12)	-0.04 (-0.17–0.09)	
Log SDV				0.057
P	0.17 (0.05–0.29)*	0.11 (0.01–0.20)	-0.02 (-0.11–0.07)	
M	-0.06 (-0.17–0.05)	0.06 (-0.20–0.08)	-0.10 (-0.20– -0.01)	
DISP R-E*				<0.05
P	3.57 (1.42–5.71)**	2.70 (1.38–4.01)**	0.04 (-1.01–1.10)*	
M	-1.01 (-2.62–0.61)	-1.05 (-2.40–0.31)	-1.46 (-2.57– -0.34)	

Data are presented as mean differences (95% confidence intervals from measurements performed 3 h after baseline (B1)). FEV_1 % pred: forced expiratory volume in one second per cent predicted; *R*rs: total resistance of respiratory system; P_{a,O_2}: arterial oxygen tension; P_{a,CO_2}: carbon dioxide arterial tension; P_{Aa,O_2}: alveolar-arterial oxygen tension difference; Log SDQ: dispersion of blood flow distribution (dimensionless); Log SDV: dispersion of alveolar ventilation (dimensionless); DISP R-E*: dispersion of retention minus excretion inert gases corrected for dead space (dimensionless). 1 mmHg=0.133 kPa. *: $p<0.05$ for comparison with placebo; **: $p<0.01$; ***: $p<0.001$; #: $p<0.0001$.

exhibited mild hypoxaemia (79 mmHg) both at 5 and 15 min after LTD_4 inhalation (table 2; fig. 1). P_{a,O_2}, P_{Aa,O_2}, Log SDQ, Log SDV and DISP R-E* values remained fairly stable throughout the whole study, such that overall LTD_4-induced decline in P_{a,O_2} was inhibited by 80 and 75% at 5 and 15 min, respectively. Changes in the four most characteristic variables (FEV_1, *R*rs, P_{a,O_2} and DISP R-E*) after montelukast or placebo pretreatment before (B1) and at 5, 15 and 45 min after LTD_4 challenge (expressed as percentage of change from B1) are depicted in figure 2. Furthermore, at 45 min both in FEV_1 (placebo: 89±4% pred; montelukast: 101±1% pred; $p<0.001$) and *R*rs (placebo: 6.7±0.8 cm $H_2O \cdot L^{-1} \cdot s^{-1}$; montelukast: 4.8±0.5 cm $H_2O \cdot L^{-1} \cdot s^{-1}$; $p<0.05$) changes still remained slightly abnormal (table 2) in the placebo arm. By contrast, P_{a,O_2} was not different between each pretreatment (placebo: 93±3 mmHg; montelukast: 97±4 mmHg), the latter finding indicating that the difference in DISP R-E* (placebo: 4.99±1.33; montelukast: 2.95±0.52; $p<0.05$) was probably marginal.

DISCUSSION

This study shows, for the first time, that in patients with mild asthma, the LTRA montelukast, at a single oral dose of 40 mg, effectively inhibits both bronchoconstriction and pulmonary gas exchange disturbances provoked by inhaled LTD_4. The current study confirms previous findings [8] that LTD_4 inhalation is followed not only by bronchoconstriction, but also by profound pulmonary gas exchange disturbances. This is reflected by mild-to-moderate hypoxaemia and marked V'_A/Q' abnormalities, very similar to those elicited in patients with natural asthma attacks. Although it is well established that montelukast and related $CysLT_1$ receptor antagonists inhibit LTD_4-induced airway narrowing [21], before the present study it was not known whether $CysLT_1$ antagonists also protected against the gas exchange defects induced by LTD_4 challenge. In view of the known presence of additional receptors for CysLTs [9], it was hypothesised that specific pulmonary vascular effects of LTD_4 would be less sensitive to the current class effects of LTRA. As there is no $CysLT_2$ receptor antagonist available for human studies, it is not possible to test the positive hypothesis, which would be to block $CysLT_2$ receptor before investigating the effects on pulmonary gas exchange of LTD_4 challenge. Alternatively, by testing the negative hypothesis the present study convincingly refuted this contention and represents, therefore, the first evidence that all lung function abnormalities provoked by inhaled LTD_4 in patients with asthma are triggered by activation of $CysLT_1$ receptors.

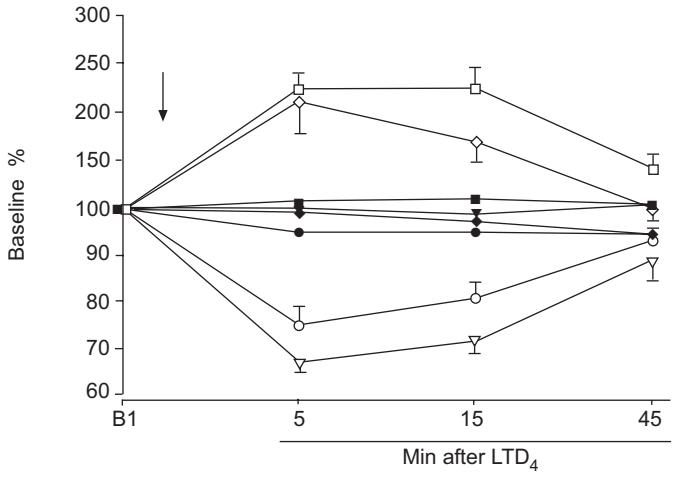

FIGURE 2. Time courses (mean±SEM) expressed as a percentage change 3 h after baseline (B1) and 5, 15 and 45 min after leukotriene D_4 (LD_4). □: respiratory system resistance (*R*rs) with placebo interventions; ◇: ventilation perfusion mismatch (DISP R-E*) with placebo intervention; ○: arterial oxygen tension (P_{a,O_2}) with placebo intervention; ▽: forced expiratory volume in one second (FEV_1) with placebo intervention; ■: *R*rs with montelukast intervention; ◆: ventilation perfusion mismatch (DISP R-E*) with monteleukast intervention; ●: P_{a,O_2} with montelukast intervention; ▼: FEV_1 with montelukast intervention. Arrow represents LD_4 challenge. See Results section for p-values.

Mechanistically, the observed effects of montelukast either mean that the gas exchange disturbances produced solely by inhaled LTD_4 are an exclusive consequence of the bronchoconstriction, or that, alternatively, $CysLT_1$ receptors in addition to initiating intense bronchoconstriction are also involved in the effects on targets other than airways smooth muscle. Although the first alternative may be the most likely, it should be admitted that activation of $CysLT_1$ receptors is established to trigger the release of other biologically active substances, such as thromboxane A_2 and prostacyclin [22–24], which may also contribute to alveolar ventilation to pulmonary blood flow imbalance by modulating pulmonary vascular tone. In fact, responses to CysLTs are enhanced in human pulmonary arteries after inhibition of prostaglandin formation [11], thus, supporting the presence of feed-back mechanisms and cascades set-up by the initial stimulation provoked by LTD_4. It has previously been shown that, at a similar degree of airflow obstruction (~30% fall in FEV_1), both histamine and MCh challenges produce similar pulmonary gas exchange abnormalities to those shown after LTD_4. Even though direct comparisons between LTD_4 challenge and MCh or histamine challenges were not performed, the results of the present study reinforce the notion that pulmonary gas exchange disturbances are nonspecific as the mechanisms of bronchoconstriction are similarly heterogeneous and mediated in both large and small airways, irrespective of the class of bronchoprovocative agent used [25]. Although it is generally held that gas exchange impairment in bronchial asthma is essentially induced by small airways, experimental models have shown that the $V'A/Q'$ imbalance becomes much more perturbed with central airway narrowing [7].

Irrespective of the ultimate mechanisms involved, the evidence of overall inhibition of LTD_4 respiratory responses by montelukast may explain, at least in part, the recently reported remarkable therapeutic efficacy shown by intravenous montelukast in acute asthma [6]. Thus, the beneficial effects of montelukast in the emergency room setting may relate not only to inhibition of the bronchoconstriction, but also to improvement of gas exchange abnormalities occurring during asthma attacks that, unfortunately, were not assessed [7]. However, it should be pointed out that the inhibition of LTD_4 effects in the authors' laboratory-human model of acute asthma cannot be necessarily applicable to the setting of naturally occurring acute asthma, where the abnormal release of LTs is previous to the administration of the LTRA. Although β_2-agonists are highly effective bronchodilators, they can associate mild-to-moderate deleterious gas exchange effects by inducing either vasodilatation or hypoxic vasoconstriction release, or both, hence making functional adaptation of the pulmonary vasculature to alveolar ventilation amelioration induced by bronchodilation less beneficial [26]. It is well established that CysLTs are final common mediators of airway obstruction induced by a large number of trigger factors in asthma, such as allergen, exercise, cold air and exposure to nonsteroidal anti-inflammatory drugs in aspirin-intolerant individuals [4]. Moreover, it has been shown that for exercise and in particular for allergens, LTRAs block the major part of the bronchoconstriction [1–4]. In allergen-induced airway obstruction in asthmatic patients, this predominant protection by LTRAs is expressed both during the early and the late inflammatory phases of the allergenic response [1]. Indeed, urinary excretion of LTE_4 is increased in acute severe asthma [27]. Moreover, *in vivo* LT biosynthesis is not inhibited by high doses of systemic glucocorticosteroids [28–30], thereby strengthening the indications to antagonise LT release during acute asthma attacks. Interestingly, in another human model for acute severe asthma, platelet-activating factor (PAF) bronchoprovocation caused its effect predominantly through the release of CysLTs as pretreatment with the 5-lipoxygenase inhibitor zileuton [31] or the LTRA zafirlukast [32] partially inhibited most of the major functional components of the response to PAF.

In conclusion, the current findings show that a cysteinyl-leukotriene receptor antagonist, such as an oral montelukast, confers a comprehensive inhibition of both bronchoconstriction and gas-exchange defects, induced by inhaled leukotriene D_4 provide strong evidence that the cysteinyl-leukotriene$_1$ receptor has a central role in the pathobiology of acute pulmonary responses mediated by cysteinyl-leukotrienes. Accordingly, this data prompts the view to extend investigations of the role of cysteinyl-leukotrienes in acute severe asthma, including life-threatening status asthmaticus. In view of the remarkable protection that montelukast and other cysteinyl-leukotreiene$_1$ antagonists offer against trigger-factor induced bronchoconstriction, it might be that more emphasis should be directed towards this particular use of leukotriene antagonists in asthma attacks caused, *e.g.* by seasonal or perennial exposure to allergens and other environmental factors that may precipitate worsening of asthma.

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