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Lack of short-term effect of the thromboxane synthetase inhibitor UK-38,485 on airway reactivity to methacholine in asthmatic subjects

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ABSTRACT: Previous open studies have suggested that thromboxane receptor antagonists or synthesis inhibitors lower airway hyperresponsiveness in human subjects. This would indicate a role of thromboxane A_2 in the development or maintenance of hyperresponsiveness in asthma.

Ten nonsmoking asthmatics (aged 23–64 yrs, 9 male) were included in a randomized, double-blind, placebo-controlled, cross-over study of the effect of one week of treatment with a potent selective thromboxane synthetase inhibitor (UK-38,485, 600 mg daily) on airway responsiveness. The study was preceded by a two week run-in period, and two weeks were used for wash-out between the two trial periods.

Adequacy of dosage and patient compliance was confirmed by a reduction in the *ex vivo* formation of thromboxane B_2 (median concentration 3.22 $\mu g \cdot ml^{-1}$ after placebo, 0.10 $\mu g \cdot ml^{-1}$ after UK-38,485, $p<0.05$). The mean forced expiratory volume in one second (FEV_1) after UK-38,485 was 2.55 *l*, compared to 2.56 *l* after treatment with placebo ($p=0.74$). The geometric mean provocative dose of methacholine producing a 20% fall in FEV_1 (PD_{20}) before and after UK-38,485 was 23.9 and 32.2 μg, respectively, compared to 25.1 and 26.3 μg respectively, before and after placebo ($p=0.31$).

The results of this study suggest that thromboxane A_2 does not play an important role in the maintenance of increased airway responsiveness in moderately severe asthmatics treated with low doses of inhaled steroids.

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Increased airway responsiveness (AR) to nonspecific stimuli is one of the fundamental characteristics of asthma. One of the most reproducible and widely used nonspecific challenge agents for demonstration of AR is methacholine, which can be safely administered by inhalation, under careful control in the laboratory setting [1, 2]. Inhaled allergens and occupational agents which cause exacerbations of asthma have been shown to increase AR [3], and, in general, the degree of AR correlates with asthma severity. The relationship between asthma activity and AR has prompted an intensive investigation into the pathogenesis of airway responsiveness, and a search for novel pharmacological agents capable of modifying AR in animals or human subjects.

Allergen challenge studies have demonstrated that a variety of lipid mediators are released from human lungs during the bronchoconstrictor response, including prostaglandin (PG) D_2, leukotriene C_4 and thromboxane A_2 (TXA_2) [4]. COLEMAN and SHELDRICK [5] demonstrated that the bronchoconstrictive potency of U46619, a stable TXA_2 mimetic, on human bronchial muscle is over 300 times as great as that of PGD_2 or $PGF_{2\alpha}$ on a molar basis [5], emphasizing the potential importance of thromboxane in asthmatic bronchoconstriction.

In a dog model, airway hyperresponsiveness can be induced by the inhalation of TXA_2 mimetic U46619 [6]. Furthermore, in this dog model, the induction of airway responsiveness after exposure to ozone could be inhibited by the administration of indomethacin [7], or by a thromboxane synthetase inhibitor OKY046 [6]. The evidence to support an important role for TXA_2 in the pathogenesis of increased baseline AR in asthmatic subjects is based on studies from Japan, in which OKY046 (a thromboxane synthetase inhibitor) [8–10], and AA_2414 (a thromboxane receptor antagonist) [11], lowered AR to methacholine in human subjects.

The purpose of this study was to investigate the effect of thromboxane synthetase inhibition on baseline AR in moderately severe asthmatic subjects, using a highly selective and potent thromboxane synthetase inhibitor, UK-38,485. The effect of 7 days treatment with UK-38,485 on baseline AR was compared with placebo, in a dosage sufficient to virtually eliminate circulating TXB_2, the main metabolite of TXA_2.

Table 1. – Baseline characteristics

Pt	Age yrs	Sex	FEV_1 *l*	FEV_1 % pred	Beclomethasone† $\mu g \cdot day^{-1}$
RF	64	M	2.07	72	100
DD	23	M	3.32	66	100
NR	61	M	2.04	73	400
BH	50	M	2.80	83	400
MP	29	M	3.54	95	400
NW	45	M	2.70	66	400
GP	24	M	3.80	90	400
JW	33	M	2.30	62	100
MB	23	M	3.50	84	200
JO	52	F	1.97	62	400

†: regular therapy with inhaled beclomethasone for at least 2 months prior to the study. FEV_1: forced expiratory volume in one second.

Patients and materials

Ten, nonsmoking asthmatic subjects (aged 23–64 yrs, 9 male) were studied. The mean baseline forced expiratory volume in one second (FEV_1) was 2.92 *l* (table 1). Four subjects were atopic, as judged by at least one positive result on skin prick testing to at least six common aeroallergens. All the atopic individuals were studied out of season, and patients taking non-steroidal anti-inflammatory agents or oral prednisolone were excluded. All subjects were using inhaled beta-agonists, on an "as needed" basis, and all subjects were regularly inhaling beclomethasone, in doses between 100–400 μg daily. The study was approved by the Newcastle Ethics Committee, and all subjects gave informed consent.

Methods

A randomized, double-blind, placebo-controlled, crossover study design was used to compare the effect of UK-38,485 and placebo on baseline pulmonary function and AR to methacholine. Following an initial 2 week run-in period, to establish stability of pulmonary function and AR, subjects were randomly allocated to active and placebo treatment groups. During the first treatment period the subjects took either UK-38,485 200 mg tablets, or identical placebo tablets, three times daily for 7 days. The provocative dose of methacholine producing a 20% fall in FEV_1 ($PD_{20}FEV_1$,) was then measured before and after a 2 week wash-out period. The subjects then switched to the alternative treatment for a further week before the final measurement of AR.

Pulmonary function

In the laboratory, spirometric measurements were made using a rolling seal spirometer (PK Morgan, Gillingham, UK). The best of five measurements of FEV_1 and forced vital capacity (FVC) was recorded at baseline and at all subsequent visits. All subjects were issued with a Wright mini peak flow meter and asked to record three measurements of peak flow, on rising in the morning and at mid-evening prior to using their standard bronchodilators. The best of each set of three was used for subsequent analysis. They were asked to keep a record of asthmatic symptoms and use of bronchodilators in a diary card.

Ex vivo *formation of thromboxane B_2*

Blood samples were taken immediately prior to the methacholine challenge test at the end of each treatment period. The blood was incubated in a water bath for one hour at 37°C. The serum was then aspirated and frozen at -40°C until measurement of TXB_2, which was performed blind, using a radioimmunoassay method as described previously [12]. The threshold of detection with the monoclonal antibody used is 0.1 $ng \cdot ml^{-1}$. The potency of UK-38,485 was established against a microsomal enzyme preparation of human platelets. Its selectivity was suggested by analysis with the cyclooxygenase enzyme and PGI_2 synthetase.

Methacholine challenge

At each visit, all subjects underwent inhalation provocation testing with methacholine, using a previously validated dosimeter technique [2]. Inhaled bronchodilators were withheld for 18 h prior to methacholine challenge, which was carried out in mid-afternoon on each occasion, using a dose range of 3–6,400 μg of methacholine. Three sets of five FEV_1 measurements were recorded at 5 min intervals, and the mean of each set calculated. The mean of the three values was used as baseline for PD_{20} calculation. Incremental doubling doses of methacholine were delivered by a calibrated dosimeter at 5 min intervals, with six measurements of FEV_1 recorded each time. The mean of the highest three FEV_1s was used. The airway responsiveness was expressed as the provocative dose of methacholine producing a 20% fall in forced expiratory volume in one second ($PD_{20}FEV_1$). Subjects in whom the second PD_{20} differed from the initial one at commencement of the run-in period by more than a doubling dose were excluded from the study.

Statistical methods

Parameters of pulmonary function (FEV_1, peak expiratory flow rate (PEFR)) were subjected to a parametric analysis of variance. The change in PD_{20} and *ex vivo* formation of TXB_2 during the active and placebo treatment periods were compared by a two way analysis of variance, and p values <0.05 were considered significant.

Results

Pulmonary function

There was no significant difference between the mean PEFR measurement or between the mean am-pm PEFR difference in the active and placebo treatment groups (table 2). The change in FEV_1 during the treatment periods is shown in table 2. There was no significant

Table 2. – Effect of UK-38,485 *versus* placebo on the mean peak expiratory flow rate (PEFR), change in forced expiratory volume in one second (FEV_1) change in PD_{20} methacholine, and *ex vivo* formation of TXB_2

	UK-38,485	Placebo
PEFR $l \cdot min^{-1}$	432 (417 to 445)	438 (422 to 451)
Diurnal variation PEFR $l \cdot min^{-1}$	16 (-7 to 39)	38 (14 to 60)
Change in FEV_1 l	-0.06 (-0.30 to 0.17)	-0.06 (-0.34 to 0.23)
Change in log PD_{20} µg	0.13 (-0.3 to 0.52)	0.02 (-0.17 to 0.21)
% of *Ex vivo* formation TXB_2 $\mu g \cdot ml^{-1}$	0.10* (0.03 to 0.22)	3.22 (2.1 to 5.45)

Data for PEFR, FEV_1, and log are mean and 95% confidence intervals in parenthesis. Data for TXB_2 are median and range in parenthesis. *: $p<0.05$, all other variables nonsignificant by analysis of variance. PD_{20}: provocative dose of methacholine producing a 20% fall in FEV_1; TXB_2: thromboxane B_2,

difference between the two treatments (p=0.98). The data relating to symptoms and bronchodilator usage was not subjected to statistical analysis, as the diary cards were incomplete in two subjects.

Airway responsiveness to methacholine

The geometric mean PD_{20} methacholine before and after UK-38,485 was 23.9 and 32.2 µg, respectively, compared to 25.1 and 26.3 µg, respectively, before and after placebo. The changes in log PD_{20} methacholine during the two treatment periods are shown in table 2. There was no significant difference between UK-38,485 and placebo.

Ex vivo *formation of thromboxane B_2*

The levels of thromboxane B_2 formed *ex vivo* at the end of each treatment period are shown in table 2. The levels of TXB_2 were significantly lower at the end of the active treatment period, compared to the placebo period (median concentration 3.22 $\mu g \cdot ml^{-1}$ following placebo treatment, 0.10 $\mu g \cdot ml^{-1}$ after active period, $p<0.05$).

Discussion

We have been unable to demonstrate that a potent and specific thromboxane synthetase inhibitor, UK-38,485, has any significant effect on either baseline FEV_1, variation in PEFR, or AR in asthmatics. It appears highly unlikely that the drug failed to inhibit thromboxane formation *in vivo*, because there was almost complete inhibition of *ex vivo* formation of TXB_2 (a non-enzymatically formed indicator of thromboxane A_2 production) after one week of treatment with UK-38,485. Our data does not support a significant role for TXA_2 in the maintenance of increased AR or bronchoconstriction in this group of asthmatics.

Advances in understanding of prostanoid metabolism have inspired optimism that novel therapies for asthma may be developed by inhibiting the synthesis of, or blocking the contractile muscle receptors for prostanoids. Several thromboxane synthetase inhibitors and receptor antagonists have now been developed, but evidence of efficacy is conflicting. Although some studies have shown elevated levels of serum TXB_2 in acute asthma, others have either failed to do so, or have raised the possibility of platelet activation in acute asthma [13, 14]. There is also evidence that inhibition of thromboxane production may result in the redirection of arachidonic acid metabolism into the production of bronchodilator prostanoids, such as prostacyclin [15], and prostaglandin E_2 [16]. However, the fact that TXA_2 receptor antagonists produce inhibition of the early asthmatic response to inhaled allergen at a similar magnitude to TXA_2 synthetase inhibition, [16–18], suggests that the effect of this shunting may be negligible.

The use of stable endoperoxides that mimic most of the actions of TXA_2 (U-44069 and U-46619) in animals or on isolated muscle tissue has confirmed its bronchoconstrictive potency [5]. In human subjects, a specific TXA_2 receptor antagonist GR32191 antagonized the bronchoconstrictive effect of TXA_2, PGD_2 and $PGF_{2\alpha}$ to a similar degree, and attenuated the early response to inhaled allergen [18]. However, in other studies GR32191 and other TXA_2 receptor antagonists have failed to inhibit the bronchoconstriction induced by exercise [19], or the inhalation of platelet activating factor (PAF) [20]. Furthermore, pretreatment with a thromboxane synthetase inhibitor CGS 12970 had no effect on the early or late reaction to inhaled allergen [21].

The evidence to support an important role for TXA_2 in the pathogenesis of increased baseline AR in asthmatic subjects is based on open studies in which a TXA_2 synthetase inhibitor OKY046 [8, 9], and a receptor antagonist AA_2414 [11], lowered AR to methacholine in human subjects. Other investigators have, so far, failed to reproduce these findings using other TXA_2 receptor antagonists [20–22] or synthetase inhibitors [23].

In contrast to the studies by FUJIMARA and co-workers [8] using OKY046, we found no evidence to support a beneficial effect of oral UK-38,485 on spirometry or airway responsiveness in asthmatics. The duration of the study was similar to that used by Fujimara and co-workers, and effective inhibition of TXA_2 synthesis was evidenced by the significant reduction in serum TXB_2 levels following treatment with UK-38,485. Although it is possible that a longer period of treatment would have resulted in a change in AR, a change in spirometric parameters would have been expected in the short-term. The reasons for the differences between the results of this study and those of Fujimara and co-workers are not clear, but may be related to differences in asthma severity or treatment, or design of the study. It is possible that the TXA_2 pathway is of less significance in asthmatic subjects on regular inhaled corticosteroids, which could in theory have removed all thromboxane-related influences. Even if this

was the case, the result of this study suggests that thromboxane has only minimal effects, and that UK-38,485 is unlikely to provide additional benefits to patients already taking low dose inhaled corticosteroids. Such drugs are unlikely, therefore, to be clinically useful. Our conclusions are further supported by a similar study in this laboratory using a thromboxane receptor antagonist GR32191, which also failed to demonstrate any effect on AR in asthmatic subjects [24].

In summary, the results of this study would suggest that thromboxane A_2 probably has, at most, a minor role to play in the variability in airway calibre and increased AR characteristic of asthma, and it is, therefore, unlikely that drugs directed against this prostanoid will prove to have a significant role in the treatment of asthma.

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