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Effect of the $K^+_{(ATP)}$ channel opener, KCO912, on baseline and allergen induced airway hyperresponsiveness in allergic rabbits

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Abstract

The effect of the adenosine triphosphate sensitive K^+ (K_{ATP}) channel opener (3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(2-oxo-1-piperidinyl)-*N*-phenyl-1-benzopyran-6-sulphonamide (KCO912) on airway hyperresponsiveness induced using either a combination of allergen immunization (i.p.) followed by aerosol allergen challenge or immunization alone was investigated. Rabbits were immunized with *Alternaria tenuis* for the first 3 months of life. Airway responsiveness to histamine was measured 24 h before and after *A. tenuis* aerosol challenge. Fifteen minutes before the second challenge, rabbits were pre-treated with 10 μg of KCO912 or vehicle by inhalation. Allergen challenge induced airway hyperresponsiveness in vehicle pre-treated rabbits and pre-treatment with KCO912 abolished the airway hyperresponsiveness. The effect of KCO912 (10 μg) or vehicle on baseline airway hyperresponsiveness to the adenosine A_1 receptor agonist, cyclopentyl adenosine (CPA), induced by immunization with *A. tenuis* alone, was also assessed. Rabbits, immunized with *A. tenuis* alone, exhibited baseline airway hyperresponsiveness as demonstrated by an increase in airway resistance to CPA. Treatment with KCO912 did not alter the allergen-induced airway responsiveness to CPA. This study demonstrates that KCO912 can inhibit allergen-induced exacerbations of airway hyperresponsiveness.

Keywords: *Alternaria tenuis*; Airway hyperresponsiveness; (Rabbit)

1. Introduction

Airway hyperresponsiveness is an important characteristic feature of asthma. It is defined as an exaggerated sensitivity and reactivity of the airways to numerous chemically unrelated compounds (Sterk and Bel, 1989). Several cell types have been implicated in the genesis of airway hyperresponsiveness, including eosinophils, mast cells, T-helper cells and B-cells (Djukanovic et al., 1990). There is evidence to suggest that multiple pathways are involved in the expression of this asthma trait (Morley, 1994; Smith and Mcfadden, 1995; Hogan et al., 1998; Van Schoor et al., 2000). Both clinical and experimental evidence point to the existence of two types of airway hyperresponsiveness; baseline airway hyperresponsiveness and exacerbations of airway hyperresponsiveness following exposure to external inciting agents such as allergens, viral infections and certain pollutants. The former is characterized by a difference of 10–100 fold in airway responsiveness between asthmatic and normal individuals to directly acting spasmogens such as histamine and methacholine and the appearance of airway responses to indirect acting stimuli such as inhaled adenosine that do not elicit changes in healthy individuals (Van Schoor et al., 2000). Over and above this underlying baseline airway hyperresponsiveness, transient increases in airway hyperresponsiveness may take place during asthma exacerbations for example following exposure to allergen, with a magnitude of about a 3–7 fold further increase (Dutoit et al., 1987; De Baets et al., 1990), which is suppressed by a number of anti-asthma drugs, particularly glucocorticosteroids (Dutoit et al., 1987; De Baets et al., 1990). However, persistent airway hyperresponsiveness remains even after chronic treatment with glucocorticosteroids (Lundgren et al., 1988) and there remains a need for novel drugs for the treatment of this phenomenon and one

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potential novel drug class are K_{ATP} channel openers (Buchheit and Fozard, 1999; Fozard and Manley, 2001).

Studies have shown that activation of these K^+ channels through K_{ATP} channel openers can induce membrane hyperpolarization particularly in airway smooth muscle and airway nerves making them less excitable to stimuli. Opening of these K^+ channels has been reported to have beneficial effects on the airways including reduction in C-fibre mediated effects (Fox et al., 1997), decreased mucus hypersecretion (Ramnarine et al., 1998), anti-tussive effects (Poggioli et al., 1999) and decreased airway hyperresponsiveness (Buchheit and Hofmann, 1996). A recent study has indeed reported that the $K^+_{(ATP)}$ channel opener, (3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(2-oxo-1-piperidinyl)-*N*-phenyl-1-benzopyran-6-sulphonamide (KCO912), inhibited increases in airway responsiveness induced by immune complexes, ozone, lipopolysaccharide and salbutamol (Buchheit et al., 2002). Based on this evidence, it has been predicted that K^+ channel openers may have some beneficial action in respiratory conditions such as asthma and chronic obstructive pulmonary diseases.

In the present study, we have used an allergic rabbit model, displaying both transient and baseline hyperresponsiveness (Herd and Page, 1996), to investigate the effects of KCO912 on both types of airway hyperresponsiveness using histamine (Herd et al., 1995) and cyclopentyl adenosine (CPA) (Ali et al., 1994; El-Hashim et al., 1999) as markers of airway hyperresponsiveness.

2. Materials and methods

2.1. Immunization protocol

The methods described in this study were subject to UK Home Office approval and performed under the Animal (Scientific Procedures) Act, 1986. The immunization of neonatal New Zealand white rabbits (NZW) rabbits was performed as described previously (El-Hashim et al., 1999). Allergen immunized rabbits were injected on the day of birth with 0.5 ml (i.p.) of *Alternaria tenuis* extract at 40,000 protein nitrogen units (PNU) ml^{-1} in aluminium hydroxide gel adjuvant and 0.9% sterile saline in the ratio of 2:1:1. The i.p. administration of allergen was repeated weekly for the first month and then biweekly for the remaining 2 months.

2.2. Pulmonary function measurement

Rabbits were prepared for measurement of pulmonary function at 3 months of age, 4–7 days after their last intraperitoneal injection of *A. tenuis*. Rabbits were sedated with diazepam (2.5 mg/kg, i.p.) and then anaesthetized with Hypnorm (0.4 ml kg, i.m.; a mixture of fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml) as previously described (El-Hashim et al., 1996).

2.3. Allergen challenge

At 3 months of age, rabbits were challenged with inhaled *A. tenuis* extract in 0.9% saline (20,000 PNU/ml) using a De Vilbiss nebuliser as previously described (El-Hashim et al., 1997).

2.4. Airway responsiveness to histamine

Airway responsiveness to histamine was determined by performing a cumulative dose–response curve to inhaled histamine (1.25–160 mg/ml; 2 min per dose) after establishing baseline response to the vehicle (0.9% saline for 2 min). Aerosols were generated with a DeVilbiss nebulizer giving particle sizes of 0.5–5 μm and were administered directly to the lungs via the endotracheal tube. Following each 2-min aerosol of histamine, approximately 10 breaths were recorded and the mean value of R_L was calculated.

2.5. Airway responsiveness to CPA

Airway responsiveness to CPA was determined by performing a cumulative dose–response curve to inhaled CPA (0.078–10 mg/ml ; 2 min per dose) after establishing the baseline response to the vehicle (50% ethanol for 2 min). Aerosols were generated with a turbo turret jet nebulizer giving particles sizes of 0.3–3 μm administered directly to the lungs via the endotracheal tube. Following each 2-min aerosol of CPA, approximately 10 breaths were recorded and the mean value of R_L was calculated. We used different nebulizers for CPA and histamine because different vehicles were used to dissolve the compounds. The vehicle for CPA (50% ethanol) is relatively more viscous than that for histamine (saline). A jet nebulizer was used to deliver CPA because these nebulizers are more efficient in delivering solutions with high viscosity than ultrasonic nebulizers.

2.6. Bronchoalveolar lavage

Bronchoalveolar lavage was performed after the determination of airway responsiveness to histamine both pre- and post-challenge with allergen as described previously (Herd et al., 1994). Cells were stained with a combination of haemotoxylin and chromotrope 2R. At least 200 cells were differentiated as either neutrophils, eosinophils or mononuclear cells based on standard morphological criteria and expressed as absolute cell counts/ml lavage fluid.

2.7. Experimental protocol 1: effect of KCO912 on airway hyperresponsiveness induced by a combination of intraperitoneal immunization followed by allergen challenge via the inhaled route

Day 1: Assessment of airway responsiveness to histamine + bronchoalveolar lavage.

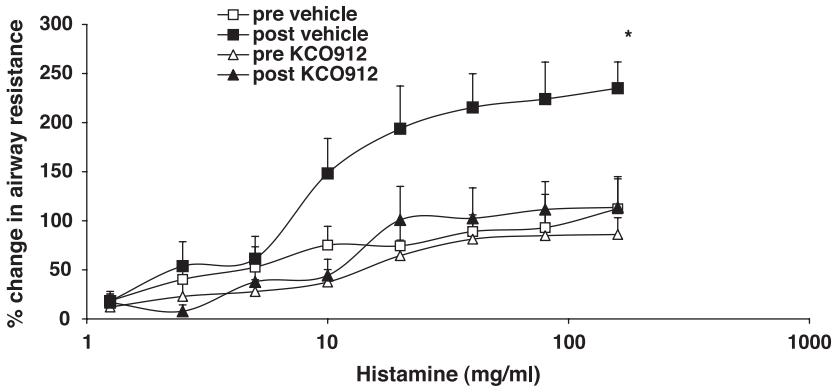

Fig. 1. Percentage change in airway resistance in response to histamine 24 h prior to and post-allergen challenge in both vehicle and KCO912 pre-treated rabbits. Values are means ± S.E.M.; $n = 8–9$. *Indicates $P < 0.05$ vs. pre-allergen (paired t-test).

Day 2: Challenge of immunized rabbits, by the inhaled route, with *A. tenuis.*

Day 3: Animals were given 10 μg of KCO912 ($n = 8$) or vehicle ($n = 9$) delivered by a jet nebulizer over 10 min. Fifteen minutes after drug or vehicle treatment, airway responsiveness to histamine was assessed and bronchoalveolar lavage was performed.

2.8. Experimental protocol 2: effect of KCO912 on baseline airway hyperresponsiveness to CPA induced by immunization alone

Group 1: Rabbits were treated with the vehicle ($n = 9$), given by aerosol, and 15 min later airway responsiveness to CPA was determined.

Group 2: Rabbits were treated with KCO912 ($n = 8$), given by aerosol, and 15 min later airway responsiveness to CPA was determined.

2.9. Materials

All reagents were of analytical grade. Drugs and chemicals used were histamine diphosphate (Sigma, Poole, Dorset, UK); CPA (Semat Technical St. Albans, Hertfordshire, UK); *A. tenuis* was obtained from Greer laboratories. Lenoir, NC, USA (40, 000 PNU/ml); aluminium hydroxide moist gel was from FSA Laboratory Supplies, Loughborough, UK); fentanyl citrate (Hypnorm, Janssen Pharmaceutical, UK); chromotrope 2R, Erhlichs Haematoxylin (Sigma diagnostics, Dorset, UK); KCO912 was supplied by Novartis Pharma, Basel, Switzerland. Male and female New Zealand White (NZW) rabbits (Froxfield Farms, Petersfield, Hamshire) were used throughout the study.

2.10. Expression and analysis of results

Airway responsiveness to inhaled histamine or CPA is expressed as the maximum % change in airway resistance ($R_{L\ max}$) from baseline values in response to increasing doses of the inhaled agonists. A Wilcoxon rank test was used to compare differences in histamine $R_{L\ max}$ values pre- and post-allergen challenge. An unpaired student t-test to compare differences in histamine $R_{L\ max}$ post-allergen challenge and CPA $R_{L\ max}$ between the two treatment groups. Cell counts, before and after allergen, were compared by a paired t-test. Cell counts, between different treatments, were compared by an unpaired t-test. A P value less than 0.05 was taken as significant.

3. Results

3.1. Airway responsiveness to histamine

In the vehicle pre-treated group, allergen induced airway hyperresponsiveness to inhaled histamine, 24 h post-challenge as demonstrated by a greater than 2 fold increase in the $R_{L\ max}$ (Fig. 1; 235.1 ± 26.7 vs. 112.2 ± 32.7, $P < 0.05$). In

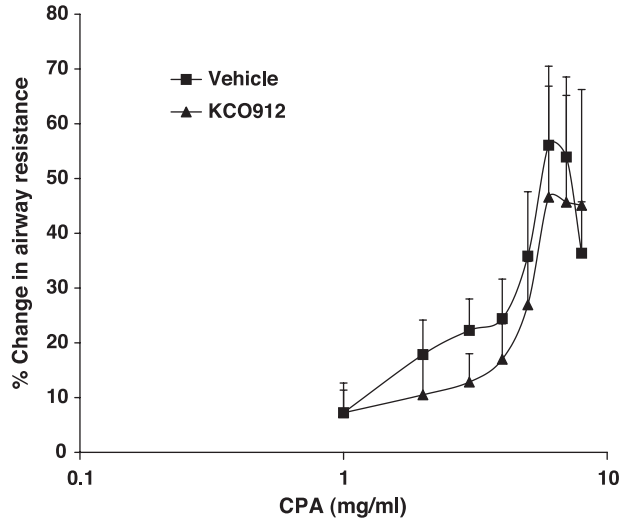

Fig. 2. Percentage change in airway resistance induced by CPA, post-*A. tenuis* immunization, in both vehicle and KCO912 pre-treated rabbits. Values are means ± S.E.M.; $n = 8–9$.

the KCO912 pre-treated group, *A. tenuis* failed to induce airway hyperresponsiveness, as there was no significant difference in between $R_{L\ max}$ pre- and post-allergen challenge (Fig. 1; 113.6 ± 29.1 vs. 86.0 ± 17.1, $P>0.05$). Furthermore, there was a significant difference in $R_{L\ max}$ between vehicle and KCO912 pre-treated rabbits post-allergen challenge (Fig. 1; 235.1 ± 26.7 *v* 86.0 ± 17.1, $P<0.05$).

3.2. Airway responsiveness to CPA

A. tenuis immunization resulted in airway hyperresponsiveness to CPA as demonstrated by dose dependent increases in airway responsiveness of both vehicle and KCO912 pre-treated rabbits (Fig. 2). There was no significant differences in airway hyperresponsiveness between KCO912 and vehicle pre-treated rabbits (45.1 ± 21.1 vs. 36.4 ± 9.4, $P>0.05$).

3.3. Bronchoalveolar lavage

The volume of fluid recovered from bronchoalveolar lavage was not significantly different between the vehicle and the KCO912 pre-treated groups before or after the allergen challenge (2.5–4.0 ml, 50–80% recovery, data not shown). The total number of leukocytes recovered in bronchoalveolar lavage fluid, 24 h after allergen challenge, was increased by more than 60% in the vehicle pre-treated rabbits (Table 1; $P<0.05$). In the KCO912 pre-treated rabbits, there was also a greater than 60% increase in total leukocyte numbers 24 h after allergen challenge (Table 1; $P<0.05$). Analysis of the differential cell counts revealed that neutrophil numbers were elevated by 8.6 fold ($P<0.05$) following allergen challenge in the vehicle treated rabbits and a similar significant ($P<0.05$) 6 fold increase in the KCO912 pre-treated group (Table 1). Eosinophils were almost undetectable before allergen challenge, in both the vehicle and KC0912 treated rabbits, but were significantly elevated following allergen exposure ($P<0.05$). There was no significant difference between KCO912 and vehicle treated rabbits in terms of total cell count, neutrophil or eosinophil numbers (Table 1; $P>0.05$).

Table 1
Total and differential cell numbers recovered from bronchoalveolar lavage fluid before and 24 h following allergen challenge in immunized rabbit pre-treated with vehicle or KCO912 via the inhaled route

	Cells ($\times 10^4$ ml^{-1} bronchoalveolar lavage)			
	Total	Neutrophils	Eosinophils	Mono's
Prior to treatment	20.9 ± 2.4	0.56 ± 0.16	0.0 ± 0.0	20.3 ± 0.16
Post-vehicle	33.4 ± 2.7[a]	4.80 ± 0.53[a]	1.5 ± 0.3[a]	27.1 ± 0.43
Prior to treatment	20.0 ± 2.5	0.96 ± 0.2	0.025 ± 0.025	19.0 ± 0.18
Post-KCO912	32.4 ± 4.2[a]	5.80 ± 0.58[a]	1.13 ± 0.25[a]	25.6 ± 0.77

Results are expressed as mean ± S.E.M. from $n=8$ rabbits.
[a] Indicates $P<0.05$ vs. pre-allergen (paired *t*-test).

4. Discussion

Inhaled glucoccorticosteroids are recommended as a first line treatment for the management of asthma (British Thoracic Society, 1993). However, there have been two major concerns regarding the use of this drug class in asthma. The first is that the side effect profile of these drugs can cause concern, particularly in the pediatric and geriatric populations. The second is that, although glucocorticosteroids are very effective in managing the transient increases in airway hyperresponsiveness observed following exposure of asthmatics to inciting agents, even after chronic therapy they do not reverse baseline airway hyperresponsiveness. Consequently, considerable effort has been made to design molecules that can effectively treat both types of airway hyperresponsiveness and have a better side effect profile, including a novel K_{ATP} channel opener, KCO912.

Data presented in the present study shows that immunization of rabbits with *A. tenuis*, from birth, results in baseline airway hyperresponsiveness to the adenosine A_1 receptor agonist CPA supporting previous data (Ali et al., 1994; El-Hashim et al., 1996, 1999). Moreover, our study confirms previous clinical and non-clinical findings that aerosol allergen challenge of allergic rabbits results in further transient exacerbations of airway hyperresponsiveness to histamine which is invariably associated with a significant eosinophilia (Gozzard et al., 1996; El-Hashim et al., 1997; Lim et al., 1999; Gauvreau et al., 2000).

We have also demonstrated that KCO912, given at a dose of 10 μg, 15 min prior to the second histamine challenge, attenuated this allergen-induced airway hyperresponsiveness compared with the vehicle treated group supporting several other studies that have shown that K^+ channel openers inhibit transient exacerbations of airway hyperresponsiveness. In the guinea-pig, it has been shown that airway hyperresponsiveness to histamine following intravenous injection of immune complexes is suppressed by treatment with either cromakalim or the benzopyran K^+ channel openers, (3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-[(3-oxo-1-cyclopenten-1-yl)oxy]-1-benzopyran-6-carbonitrile (SDZ PCO 400) (Chapman et al., 1991). Moreover, infusion of platelet activating factor in guinea pigs induces airway hyperresponsiveness to histamine that is suppressed by SDZ PCO 400 (Mazzoni et al., 1991). Also, K^+ channel openers such as bimakalim, levcromakalim and SDZ PCO 400 produced an almost complete suppression of immune-complex induced airway hyperresponsiveness to histamine (Buchheit and Hofmann, 1996). Furthermore, another KCO, SDZ 217-744 [(3*S*,4*R*)-*N*-[3,4-dihydro-2,2-dimethyl-3-hydrox-6-(2-methylpyridin-4-yl)-1-benzopyran]-3-pyridinecarboxamide] has been show to inhibit airway hyperresponsiveness induced by chronic salbutamol administration (Buchheit and Fozard, 1999). Indeed, it has recently been reported that airway hyperresponsiveness, in guinea pigs, induced through by a variety of stimuli, is suppressed by KCO912. Thus, following intra-tracheal administration,

KCO912 reversed airway hyperresponsiveness induced by immune complexes, LPS or ozone (Buchheit et al., 2002). This effect on airway hyperresponsiveness was achieved within a time frame very similar to the effect we report here in allergic rabbits. Such inhibition is not due to bronchodilatation as it has been shown that K^+ channel openers such as bimakalim, levcromakalim, SDZ PCO 400 and more recently KCO912, do not inhibit bronchoconstriction in normoreactive guinea pigs at doses that suppressed airway hyperresponsiveness (Buchheit and Hofmann, 1996; Buchheit et al., 2002). This would suggest that K^+ channel openers are not effective bronchodilators and that bronchodilation is not the mechanism for their suppression of airway hyperresponsiveness.

KCO912 did not affect allergen induced cellular influx in the airways in our study and the lack of effect on cellular recruitment into the airway was not surprising given that the drug was administered 24 h after the allergen challenge. Our results clearly suggest that it is unlikely that KCO912 is inhibiting airway hyperresponsiveness via an effect on airways cellular infiltration. However, we cannot rule the possibility that KCO912 or K^+ channel openers in general may have the potential of inhibiting cellular infiltration and/or activation if administered prior to the allergen challenge.

One potential mechanism by which KCO912 produced its suppressive effect on the allergen induced airway hyperresponsiveness is via an effect on airway nerves. Many studies have reported that airway hyperresponsiveness induced by a variety of stimuli can be inhibited by capsaicin, including allergen and platelet activating factor induced airway hyperresponsiveness in rabbits (Herd et al., 1995; Spina et al., 1991). In addition, bilateral vagotomy before treatment with immune complexes abolished the airway hyperresponsiveness to histamine in guinea-pigs (Sanjar et al., 1990). These data suggest an involvement of airway nerves in the pathogenesis of airway hyperresponsiveness and it is of interest therefore that some studies have shown that K^+ channel openers can modulate neuronal function in the airways. For example, one study in the guinea pig isolated trachea, reported that cromakalim abrogated non-adrenergic non-cholinergic (NANC), but not substance P-mediated bronchoconstriction (Ichinose and Barnes, 1990). The same study showed that contractions induced by vagal stimulation of the isolated trachea were also inhibited by cromakalim. Also, K^+ channel openers have been reported to inhibit several types of neurogenic-driven afferent and efferent effects including induced mucus secretion (Ramnarine et al., 1998), experimentally induced cough in guinea pigs, together with inhibition of C-fibre activity and eNANC mediated bronchoconstriction (Fox et al., 1997; Poggioli et al., 1999).

In marked contrast to the effects of KCO912 on allergen induced airway hyperresponsiveness, our data showed that this compound had no effect on baseline airway hyperresponsiveness to the adenosine A_1 receptor agonist CPA. In a previous study, we have also reported a lack of effect of dexamethasone on baseline airway hyperresponsiveness to CPA, even when the treatment was maintained for a period of 1 month (El-Hashim et al., 1999), suggesting that the mechanisms underlying this baseline airway hyperresponsiveness do not involve airways inflammation and hence, it follows that the mechanisms of baseline airway hyperresponsiveness to CPA are likely different to those contributing to the exacerbations of airway hyperresponsiveness. Indeed, the mechanisms underlying baseline airway hyperresponsiveness to CPA, in this model, are not really known. However, it is clear from previous studies that up-regulation of the adenosine A_1 receptor is the predominate mechanism underlying the baseline airway hyperresponsiveness to CPA (Nyce and Metzger, 1997; Ali et al., 1994). It is therefore possible that bronchoconstriction, through activation of the adenosine A_1 receptor on rabbit ASM, is what may be causing this airway hyperresponsiveness to CPA. As K_{ATP} channels openers are generally weak bronchodilators, it may therefore not be too surprising that this response was not reversed by KCO912 at the dose used.

In conclusion, with the dosing regime used, KCO912 inhibited the allergen-induced airway hyperresponsiveness independent of the existing eosinophilia, thus confirming that this type of airway hyperresponsiveness can be inhibited without reducing cellular influx.

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