Effect of oral terfenadine on bronchoconstrictor response to inhaled neurokinin A and histamine in asthmatic subjects


ABSTRACT: Neurokinin A (NKA) elicits a potent contractile effect in asthmatic airways. Its mechanism of action in bronchial asthma is still unknown. Recent work supports the view that NKA may stimulate mediator release from mast cells.

In the subjects studied, oral terfenadine, when compared to placebo, significantly increased the volume (range) of induced histamine required to reduce forced expiratory volume in one second (FEV₁) from 0.65 (0.03-0.08) mg (0.16 (0.10-0.26) µmol) to 1.19 (0.63-2.04) mg (3.88 (2.05-6.64) µmol). However, terfenadine failed to increase the airway responsiveness to NKA in all of the subjects studied, the geometric mean (range) PD_{10} NKA value being 0.94 (0.47-2.49) µg (6.36 (4.14-21.9 nanomol) and 0.75 (0.45-1.59) µg (6.62 (4.23-14.0) nanomol) after placebo and terfenadine, respectively.

We conclude that NKA is a potent bronchoconstrictor agonist in asthma, being approximately 19 times more potent than histamine in molar terms. In this study on a small number of subjects, pharmacological intervention with oral terfenadine failed to achieve significant protection of the airways against the contractile effect of NKA. Thus, it is unlikely that the bronchoconstriction provoked by inhaled NKA in asthma is mediated through histamine release from airway mast cells.

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A considerable body of work supports the view that neuropeptides, such as neurokinin A (NKA) and substance P (SP), may be implicated in the regulation of the airway tone and as inflammatory mediators in asthma [1-3]. Recently, NKA has been demonstrated in human airways and appears to be co-localized with SP; whereas NKB does not appear to be present [4]. Endogenous tachykinins, such as NKA and SP, exhibit a variety of features, such as contraction of airway smooth muscle, increased vascular permeability and mucus secretion, which may all be relevant to the pathophysiology of bronchial asthma [5]. However, final confirmation of their role as putative mediators in asthma must await demonstration of their release into the airways following allergen challenge and availability of specific neuropeptide antagonists.

The method by which endogenous tachykinins elicit smooth muscle contraction is not well understood, and may vary between species. It has been suggested that in rabbit airways the action of SP is indirect via the release of acetylcholine [5]. However, in guinea-pig trachea [6], and in human bronchi [7], cholinergic blockade with atropine was reported to have no effect on the contractile response provoked by SP. The bronchoconstrictor effect of nebulized NKA in asthmatic patients is inhibited by prior treatment with nedocromil sodium [8, 9], indicating that it is mediated indirectly, rather than via a direct effect on airway smooth muscle.

There is some support for an action of endogenous tachykinins in eliciting mast cell mediator release. Mast cells have recently been reported to be closely related to tachykinin-containing nerve endings [10]. In human dispersed skin mast cells, NKA and SP caused significant dose-dependent histamine release [11]. In support of this, FULLER et al. [12] have shown that, in man, the histamine H1-receptor antagonist, terfenadine, significantly inhibited the wheal and flare response to intradermally injected NKA. In addition, histamine release in bronchoalveolar lavage has been reported after NKA infusion in the rat in vivo [13], thus, raising the possibility that mediator secretion from mast cells may contribute to the airways response induced by NKA in asthma.

To investigate this possibility, we have assessed the relative contribution of mast cell histamine to the mechanism of NKA-induced bronchoconstriction in asthma, by undertaking
f/s ch pn e umotachograph (Pulmonary System farinae, Bronchial provocation their informed consent tory Diseases (University of Catania), and all subjects gave siveness, reflected by a provocative dose of histamine sodium cromoglycate dosimetric technique [16]. None had received oral or inhaled corticosteroids, theophylline, antihistamines or bronchodilators were withheld for at least 24 h prior to each visit to the laboratory. Subjects were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma, and all visits to the laboratory were performed in the absence of any drug treatment, subjects attended the study each month. Inhaled bronchodilators were withheld for at least 24 h prior to each visit to the laboratory. Subjects were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma, and all visits to the laboratory were carried out at the same time of day. The study was approved by the Ethics Committee of the Department of Respiratory Diseases (University of Catania), and all subjects gave their informed consent.

**Materials and methods**

**Subjects**

Six asthmatic subjects (3 males, 3 females), with a mean (±SEM) age of 31 (4.4) yrs, referred to our hospital chest clinic with stable asthma as defined by the American Thoracic Society [15], participated in the study (table 1). All subjects had a history of dyspnoea with wheezing or chest tightness after exposure to airborne allergens and were nonsmokers with positive skin prick test (>2 mm weal response) to one or more of six common aeroallergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, wall pellitory grass, mixed grass pollens, cat fur, dog hair). At the beginning of the study, all patients were asymptomatic, with a baseline forced expiratory volume in one second (FEV1) of >80% of their maximum predicted values. All subjects had evidence of bronchial hyperresponsiveness, reflected by a provocative dose of histamine resulting in a 20% fall in FEV1 (PD20) of <8 μmol with a dosimetric technique [16]. None had received oral or inhaled corticosteroids, theophylline, antihistamines or sodium cromoglycate within the previous month. Inhaled bronchodilators were withheld for at least 24 h prior to each visit to the laboratory. Subjects were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma, and all visits to the laboratory were carried out at the same time of day. The study was approved by the Ethics Committee of the Department of Respiratory Diseases (University of Catania), and all subjects gave their informed consent.

**Bronchial provocation**

The airway response was measured as FEV1, using a Fleisch pneumotachograph (Pulmonary System 47120A, Hewlett Packard Instrument), the better of the two consecutive measurements being recorded. On each study day, histamine acid phosphate (BDH Chemicals, Poole, Dorset, UK) and neurokinin A (Peninsula Laboratories) were freshly prepared in 0.9% sodium chloride and 1% albumin in 0.9% sodium chloride, respectively, to produce a range of doubling doses of 0.015–44 mg (0.005–13.02 μmol) for histamine, and of 0.32–10.44 μg (2.85–92 nmol) for NKA. To avoid peptide degradation and adherence to plastic surfaces, NKA was stored at 4°C prior to use and bronchoprovocation was performed within 30 min of reconstitution. Bronchial challenges were performed, using a slightly modified version of the dosimetric technique of Yan et al. [17]. The solutions were administered as aerosols, generated from a starting volume of 2 ml, in a nebulizer (DeVilbiss 646) driven by compressed air at 12 l/min [18]. Under these conditions, the nebulizer generates 16.7 μl for each breath. Each dose was administered via a mouthpiece, whilst wearing a noseclip, by 15 tidal breaths controlled by a dosimeter (Mefar, Brescia). The nebulized solution was generated only during inspiration time, using a microcomputer which was linked to a solenoid valve. The inhalation (0.8 s) and exhalation (1.6 s) times had been previously standardized. With this technique, the asthmatic range for histamine responsiveness is approximately 0.015–8 μmol [16].

**Study design**

The study consisted of two phases. In the first phase, in the absence of any drug treatment, subjects attended the laboratory on two separate occasions, at least 72 h apart, to undertake dose-response studies with inhaled histamine and NKA, respectively. On each occasion, after 15 min rest, three baseline measurements of FEV1 were recorded at 3 min intervals. Subjects then inhaled aerosolized 0.9% sodium chloride and FEV1 measurements were repeated at 1 and 3 min, the higher value being recorded. Provided that FEV1 did not fall by >10% of the baseline value, a bronchial provocation study was carried out with inhaled histamine or NKA. FEV1 was recorded at 1 and 3 min after administration of each dose of agonist, and increasing

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age yrs</th>
<th>Baseline FEV1 % pred</th>
<th>Atopy†</th>
<th>PD20 histamine mg</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>35</td>
<td>96</td>
<td>D, W</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>43</td>
<td>81</td>
<td>D, W</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>17</td>
<td>103</td>
<td>W, G</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>37</td>
<td>96</td>
<td>W, G</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>34</td>
<td>88</td>
<td>W</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>18</td>
<td>98</td>
<td>D</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Mean: 31; ±4.4; 94; 0.046*; ±3.2; (0.03–0.05) *

*: geometric mean (range); †: atopic, positive immediate skin test to one or more allergens; D: Dermatophagoides sp.; W: wall pellitory; G: grass; FEV1: forced expiratory volume in one second; PD20: provocation dose producing a 20% fall in FEV1.
Doubling doses of histamine or NKA were inhaled at 5 min intervals until FEV₁ had fallen by >20% for histamine and by >15% for NKA of the post-saline baseline value, so that their respective PD₂₀ and PD₁₅ values could be derived.

In the second phase of the study, subjects attended on four further occasions, at least 5 days apart, to undertake dose-response studies with inhaled histamine and NKA in a manner similar to that described for phase 1. On the first two occasions, increasing doubling doses of histamine were administered after three days treatment with either oral terfenadine (180 mg q.d.) or matched placebo, administered in a randomized, double-blind fashion. Subjects took the last set of tablets 3 h prior to bronchial challenge.

On the final two occasions, subjects undertook doseresponse studies with inhaled NKA after three days of treatment with either oral terfenadine (180 mg q.d.) or matched placebo administered in a randomized, double-blind fashion. Subjects took the last set of tablets was administered 3 h prior to bronchial challenge.

**Data analysis**

Figures refer to the mean±SEM, unless otherwise stated, and the p<0.05 level of significance was accepted. Pre- and post-treatment baseline FEV₁ values prior to bronchial challenges were compared within and between study days by two-factor analysis of variance (ANOVA). The airways response at each agonist dose was expressed as the percentage fall from the post-saline baseline FEV₁ value. The percentage decrease in FEV₁ was plotted against the cumulative dose of agonist administered on a logarithmic scale, and that dose producing a fall in FEV₁ from the post-saline baseline value (PD FEV₁) determined by linear interpolation.

The repeatability of the challenge procedure with inhaled histamine and NKA was determined, according to the method described by ALTMAN and BLAND [18] of plotting the difference against the mean of the log-transformed PD values obtained on the placebo and open study days. The mean and standard deviation of the difference between these values were then derived, and used to calculate the coefficient of repeatability (CoR) between the results of the two study days.

The PDₐ₅ and PD₁₅ values were log-transformed prior to comparison by Student's test for paired data.

The relationship between PD₁₅ histamine and the PD₁₅ NKA was investigated by least squares linear regression analysis of the log-transformed data.

Power calculations, based on the assumption that a significant rightward shift in the dose response curve for NKA is approximately one doubling dose, indicate that for the six subjects studied there is a 70% chance of detecting a significant difference with a significance level of <5% (two-sided).

**Results**

There were no significant differences in the mean baseline and post-diluent values of FEV₁ between and within study days (table 2).

For the six subjects studied, the geometric mean (range) PD₂₀ and PD₁₅ values obtained on the open study days for histamine and NKA were 0.05 (0.03-0.05) mg (0.13 (0.10-0.16) μmol), and 8.4 (5.4-18.3) nmol (0.95 (0.61-2.08) μg), respectively (table 3). The challenge procedures with

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**Table 2.** - Effects of 3 days of treatment with terfenadine (180 mg q.d) and placebo on baseline FEV₁ values

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Baseline</th>
<th>Placebo</th>
<th>Terfenadine</th>
</tr>
</thead>
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<td>3.68</td>
<td>3.95</td>
</tr>
<tr>
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<td>3.20</td>
<td>3.36</td>
<td>3.20</td>
</tr>
<tr>
<td>6</td>
<td>3.16</td>
<td>3.18</td>
<td>3.12</td>
</tr>
</tbody>
</table>

Mean: 3.14 ± 0.18; SD: 3.14 ± 0.20.

**Table 3.** - Effects of 3 days treatment with terfenadine (180 mg q.d) and placebo on airway histamine and NKA responsiveness

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Baseline</th>
<th>Placebo</th>
<th>Terfenadine</th>
<th>Baseline</th>
<th>Placebo</th>
<th>Terfenadine</th>
</tr>
</thead>
<tbody>
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</tr>
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<tr>
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<td>0.03</td>
<td>0.08</td>
<td>0.06</td>
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</tr>
<tr>
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<td>0.06</td>
<td>0.06</td>
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<td>4.1</td>
<td>4.2</td>
</tr>
<tr>
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<td>0.03</td>
<td>0.03</td>
<td>0.21</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
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<tr>
<td>6</td>
<td>0.04</td>
<td>0.04</td>
<td>0.93</td>
<td>6.1</td>
<td>6.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Geo. mean: 0.05; Range: 0.03-0.05; 0.03-0.08; 0.63-2.04; 5.4-18.3; 4.1-21.9; 4.2-14.0.

NKA: neurokinin A; PD₂₀ and PD₁₅: provocative dose producing a 20% and 15% fall in forced expiratory volume in one second, respectively; Geo. mean: geometric mean.
Fig. 1. — Effect of oral placebo (bold line) and terfenadine (dotted line) on the dose-related falls in FEV₁ produced by inhaled histamine in six asthmatic subjects. FEV₁: forced expiratory volume in one second.

Fig. 2. — Effect of oral placebo (bold line) and terfenadine (dotted line) on the dose-related fall in FEV₁ produced by inhaled NKA in six asthmatic subjects. FEV₁: forced expiratory volume in one second; NKA: neurokin A.
the two agonists were found to be repeatable, there being a CoR of 1.1 and 0.5 doubling doses for histamine and NKA, respectively.

For the six subjects studied, the geometric mean (range) PD20 histamine and PD15 NKA after placebo administration were 0.05 (0.03-0.08) mg (0.16 (0.10-0.26) μmol) and 8.4 (4.1-21.9) nmol (0.94 (0.47-2.49) μg), respectively (table 3). Thus, on a molar basis, NKA was 19 (7-49) fold more potent than histamine in provoking bronchoconstriction in this group of asthmatic subjects. A lack of correlation was reported between PD50 values histamine and NKA (p=0.19; R=0.61).

When compared with placebo, oral terfenadine produced displacement to the right of the dose-response curves obtained with histamine (fig. 1), but not with NKA (fig. 2). The geometric mean (range) PD20 and PD15 values obtained with histamine and NKA after terfenadine were 1.19 (0.63-2.04) mg (3.88 (2.05-6.64) μmol) and 6.6 (4.2-14.0) nmol (0.75 (0.48-1.59) μg), respectively (table 3). When mean (range) changes in PDs after terfenadine administration was expressed in doubling doses, we found that for histamine and NKA challenges there was a 4.6 (3.4-5.4) and -0.35 (-1.36-0.03) doubling dose change following terfenadine treatment.

Discussion

This study demonstrates that NKA administered by inhalation to asthmatic subjects causes dose-related bronchoconstriction, thus confirming the findings of previous studies with this agonist [2, 9]. In addition, we have shown that premedication with the selective histamine H1-receptor antagonist, terfenadine, failed to produce a significant protection for the airways against NKA provoked decreases in FEV1, suggesting that histamine is not involved in mediating the response with this agonist.

Terfenadine was chosen as a histamine H1-receptor antagonist because of its demonstrated potency and specificity [14]. It has been shown to displace the dose-response curve to inhaled histamine in asthmatic subjects by a mean 30 fold [19], and it is without significant effect on the bronchial response to methacholine [20], indicating a lack of affinity for muscarinic receptors. In the present study, a similar inhibition was obtained by terfenadine pretreatment, the dose-response curve to inhaled histamine being displaced by a mean 24 fold. However, in the group of asthmatics studied, oral terfenadine failed to elicit significant bronchodilation, as had been reported previously [19, 21]. Possible reasons for this discrepancy may be that our patients were completely asymptomatic at the time of the investigation, and that their baseline spirometric values were considerably higher compared with those of the other studies (mean baseline FEV1 value of the subjects studied was 94% of predicted), so limiting the potential for bronchodilation.

In our group of subjects, NKA was approximately 19 fold more potent than histamine in provoking bronchoconstriction, and there was no significant correlation between the responsiveness of the airways to these two agonists, confirming previous findings by our group and others [8, 9]. The lack of correlation observed in the present study indicates a diversity in the mode of action of these agonists, and suggests the activation of different bronchospasmodic pathways.

The mode of action of NKA in provoking bronchoconstriction in asthmatic subjects is unknown, but possibilities include a direct effect on airways smooth muscle, an interaction with neural reflexes of both the vagal cholinergic and C-fibre afferent types, mast cell mediator release, and vascular changes in the airways [3]. Although NKA and NK2-selective agonists are potent constrictors of human bronchi in vitro [4, 22-24], it is possible that the mechanism of action of NKA in asthma involves a direct contractile effect on airways smooth muscle. In human studies, nedocromil sodium, a drug which possesses the ability to inhibit C-fibre-mediated neural reflexes, in addition to its effects on inflammatory cells including mast cells [25], produced significant inhibition of decreases in pulmonary function provoked by NKA [8, 9], suggesting that this peptide may act indirectly via an interaction on nerves and/or inflammatory cells.

A recent study by LOWMAN et al. [11] has demonstrated that NKA can provoke release of histamine from human skin mast cells in vitro. Some support for this mechanism operating in vivo comes from studies in which, in man, terfenadine significantly reduced the wheal and flare responses to intradermally injected NKA, thus suggesting that histamine contributes as a mediator of NKA-induced response [12]. That the same mechanism of action may be active at the airways level is suggested by two recent investigations carried out in animal models. In the rat, mast cell activation in the NKA response has been suggested by the large inhibitory effect of methysergide on the bronchoconstriction induced by infusion with this peptide [26]. In addition, it was demonstrated that, in a rat model, histamine was present in large amounts in bronchoalveolar lavage fluid recovered after adenosine and NKA infusion [13]. Our failure to show a significant inhibitory effect of oral terfenadine on the airway response to challenge with inhaled NKA is at variance to the findings in rodents. A recent study in rhinitic subjects, involving provocation of the nasal airways with NKA, failed to demonstrate histamine release in lavage fluid [27], in agreement with the results of lack of contribution of this amine to the airways effects of NKA. In a similarly designed clinical trial, we have also shown that premedication with the potent histamine H1-receptor antagonist, astemizole, failed to protect against SP-induced bronchoconstriction [28], thus supporting our view that endogenously released histamine does not contribute as mediator of neurotropic-induced bronchoconstriction in human asthma. In the same study, the bronchospastic response to inhaled SP was weakly attenuated by the anticholinergic drug ipratropium bromide, thus indicating involvement of a cholinergic component in the response. It is possible that the lack of effect of terfenadine on NKA-induced bronchoconstriction seen in the present study may be due to the fact that local concentrations of exogenous NKA were too low to activate airway mast cells or, alternatively, that human mast cells within bronchial mucosa are unresponsive to NKA, as indicated by the recent literature on mast cells heterogeneity [29]. In addition, although in human mast cells neuropeptides are poor activators of the oxidative pathway for newly formed mediators, such as prostaglandin D2 (PGD2) and leukotriene C4 (LTC4) [29], we
cannot exclude the possibility that part of the bronchoconstrictor response to inhaled NKA in asthmatic subjects could be due to the activation of mast cells, and subsequent local generation of contractile prostaglandins and leukotrienes.

In conclusion, this study on a small number of subjects has shown that terfenadine failed to produce a significant protection of the airways against provocation with NKA, and that it is unlikely that mast cell histamine release contributes to the action of this peptide in asthmatic airways. However, final confirmation of their role as putative mediators in asthma must await demonstration of their release into the airways following allergen challenge and availability of specific neuropeptide antagonists. Further studies are required to clarify both the mode of action of this agonist and its role in bronchial asthma.

References