No effect of the oral neutral endopeptidase inhibitor, candoxatril, on bronchomotor tone and histamine reactivity in asthma

R.M. Angus, M.J.A. McCallum, J.E. Nally, N.C. Thomson

ABSTRACT: Neutral endopeptidase (NEP) is found in many tissues in man, including the lung. Metabolism by NEP is one of the main mechanisms for the clearance of atrial natriuretic peptide (ANP), a hormone that causes bronchodilation and reduces nonspecific bronchial reactivity in man. Candoxatril, an oral NEP inhibitor has been shown to elevate circulating ANP levels. We have sought to determine whether the administration of candoxatril will alter bronchomotor tone (forced expiratory volume in one second (FEV1)) and histamine reactivity.

Ten male asthmatic patients with stable asthma were enrolled (mean (SD) age 32 (10) yrs; FEV1 92 (11) % predicted) in a randomized, double-blind, placebo-controlled study. On each study day, after baseline spirometry, patients received 200 mg of candoxatril or placebo. Spirometry was repeated at half hourly intervals. After 2 h a histamine inhalation test was performed.

There was no significant difference in FEV1 values at baseline or at 2 h post-dosing between active and placebo study days, with mean (SEM) FEV1 at baseline and 2 h of 3.71(0.29) l and 3.85(0.29) l on the placebo day, and 3.89(0.27) l and 4.05(0.82) l on the active day, respectively. The geometric mean (range) provocative concentration of histamine producing a 20% fall in FEV1 (PC20) on the placebo day and active day did not differ significantly, being 1.17(0.25–25.8) and 0.93(0.13–32) mg·ml-1, respectively. ANP levels rose significantly on the active day, from a mean (SEM) baseline value of 12.1 (2.1) pg·ml-1 to 29.1(4.8) pg·ml-1 at 2 h, but did not change on the placebo day (baseline 11.54(2) pg·ml-1 and 2 h level 10.8(1.9) pg·ml-1. Plasma drug levels were maximally elevated between 2 and 3 h mean (SEM) plasma candoxatril at 1 h 161(53), 2 h 846(136), 3 h 896(68) and 4 h 626(60) ng·ml-1. No side-effects were observed or noted by the patients.

We conclude that the acute administration of the oral NEP inhibitor, candoxatril, does not alter bronchomotor tone or bronchial reactivity to histamine in stable asthmatic patients. This neutral effect of candoxatril is reassuring, and permits further exploration of NEP inhibitors in the treatment of e.g. cardiac failure.

Keywords: Atrial natriuretic peptide bronchial reactivity bronchomotor tone candoxatril histamine neutral endopeptidase inhibitor

Intravenous atrial natriuretic peptide (ANP) has been shown to cause bronchodilation and reduce bronchial reactivity in man [1–6]. When given by inhalation to asthmatic patients, ANP has been shown to attenuate histamine- and methacholine-induced contraction, and in high doses it has a small bronchodilator effect [7–9]. In vitro we have shown that ANP relaxes human bronchial smooth muscle preconstricted by methacholine, and that preincubation with ANP attenuates methacholine-induced contraction [10]. Both of these effects are significantly enhanced by the addition of the neutral endopeptidase (NEP) inhibitor, phosphoramidon [10]. ANP would appear, therefore, to have therapeutic potential in asthma. However, as it is a peptide, ANP is not orally bioavailable and inhalation studies have, so far, demonstrated only a modest effect. It is likely, therefore, that any clinical application in asthma would be limited by the need for continuous intravenous infusion and its relatively short duration of action

ANP is cleared from the plasma by two main pathways: degradation by the enzyme NEP [11] and binding to a non-guanylyl cyclase clearance receptor [12]. Neutral endopeptidase 3.4. 24.11 (NEP) is a zinc-containing metalloendopeptidase, first localized to the brush border of the kidney, in 1974 [13]. Since then, NEP has been found in many sites within the body and in a variety of species other than man [11]. In the lung, NEP appears to be present in high concentrations in airway...
epithelium, although it is also found in submucosal glands, airway smooth muscle and nerves [14]. An alternative approach to harnessing the therapeutic potential of ANP on the airway might be to systemically inhibit NEP, thereby allowing elevation of endogenous hormone levels. Candoxatril (Pfizer Central Research, Sandwich, Kent, UK) is a potent specific inhibitor of NEP, which has been shown to enhance plasma ANP concentrations [15, 16]. The present study is based on the rationale that the systemic inhibition of NEP by candoxatril might elevate airway ANP levels sufficiently to favourably affect bronchomotor tone and bronchial reactivity and to overcome the potential effects of bronchoconstrictor tachykinins, which are also metabolized by NEP.

Methods

Patients

Ten adult male, atopic asthmatic patients were recruited from the out-patient clinic (mean (sd) age 32 (10) yrs, forced expiratory volume in one second (FEV1) 92(11) % predicted) (table 1). Asthma was diagnosed in accordance with American Thoracic Society guidelines [17]. As a condition of enrolment, the patient’s FEV1 had to be ≥70% of predicted despite withholding β2-agonists. Each patient’s asthma had been stable (defined as no respiratory tract infection or deterioration of asthma control requiring an alteration in anti-asthma treatment) for at least 2 months prior to recruitment. All were taking short-acting β2-agonists, and 7 of the 10 inhaled corticosteroids.

The study had the approval of the Glasgow West Ethics Committee and informed written consent was obtained from each patient.

Study design

A randomized, double-blind, placebo-controlled study design was employed. Two study days were required. Table 1. – Patient characteristics

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Age (yrs)</th>
<th>FEV1 (l)</th>
<th>% pred</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>2.44</td>
<td>74</td>
<td>S, B*</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>2.89</td>
<td>85</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>4.79</td>
<td>112</td>
<td>S, B*</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>4.12</td>
<td>91</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>3.69</td>
<td>103</td>
<td>S, B*</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>4.62</td>
<td>87</td>
<td>T, Bu</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>3.2</td>
<td>97</td>
<td>S, B**</td>
</tr>
<tr>
<td>8</td>
<td>21</td>
<td>4.62</td>
<td>97</td>
<td>S, B*</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>3.81</td>
<td>84</td>
<td>A, B*</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
<td>4.52</td>
<td>96</td>
<td>A, B*</td>
</tr>
</tbody>
</table>

Mean (sd) 31.6 (10) 3.87 (0.8) 92 (11)

Pt: patient; A: antihistamine; Bu: inhaled budesonide; B: inhaled beclomethasone; S: inhaled salbutamol; T: inhaled terbutaline; FEV1: forced expiratory volume in one second. *: <1,000 µg beclomethasone or <800 µg budesonide; **: ≥1,000 µg beclomethasone.

and patients attended at the same time of day on both occasions. Inhaled β2-agonists were discontinued for 8 h prior to attendance on each study day but inhaled corticosteroids were continued unchanged. Patients were asked to fast, except for water, for 2 h prior to the study. If the FEV1 was within 10% of the baseline on the screening day, the study proceeded on that day. Patients were asked to lie semirecumbent and an intravenous cannula (Venflon; Viggo AB, Helsingborg, Sweden) was inserted for blood-sampling in the ante-cubital vein. Patients remained in a semirecumbent position, except for performing spirometry. Prior to dosing, heart rate and blood pressure were measured. Blood pressure and pulse were monitored every 15 min using a semi-automatic sphygmomanometer. Blood (20 ml) was sampled for drug and hormonal (ANP, cyclic guanosine monophosphate (cGMP) and catecholamines) assay at times 0, 30, 60, 90, 120, 180, 240 min. Spirometry was checked every 30 min, and at 2 h histamine inhalation test was performed using the method of Cockcroft et al. [18]. After completion of the challenge, the patient received 2.5 mg of salbutamol via a nebulizer and spirometry was repeated after 20 min. After 4 h, the patient was allowed home following completion of a physical examination.

Measurements

FEV1. This was measured using a dry wedge spirometer (Vitalograph S, Vitalograph, Buckingham, UK), the best of three readings being taken at each time-point.

Pulse and blood pressure. These were measured using a semi-automatic sphygmomanometer (Critikon: Dinamap vital signs monitor 1846 FX, Berkshire, UK). Three readings were taken at each time-point from the non-dominant arm, the mean being recorded.

Histamine inhalation test [18]. Baseline FEV1 was measured, taking the best of three readings. Thereafter, a saline inhalation was administered and then doubling doses of nebulized histamine were administered at 5 min intervals, beginning with 0.0625 mg·ml−1. Each concentration was given for 2 min via a Wright’s gas nebulizer driven by compressed air, with an output of 0.13 ml-min−1. The FEV1 was measured at 0.5, 1.5 and 3 min after each inhalation, until a fall of at least 20% was achieved. The result was then expressed as the histamine provocation concentration producing a 20% fall in FEV1 (PC20).

ANP. Venous blood (10 ml) was collected into potassium ethylenediamine tetra-acetic (EDTA) tubes containing 1,000 IU aprotinin (Bayer, Newbury, UK), stored on ice and spun within 2 h. Plasma was stored at -20°C, and ANP was later measured by radioimmunoassay following pre-extraction with C18 reverse phase columns (Sep-Pak; Waters, Milford, MA, USA). The assay has previously been described in detail [19], both inter- and intra-assay variation ≤8%.
cGMP. Venous blood (5 ml) was collected into lithium heparin tubes, stored on ice and spun within 2 h. Cyclic GMP was later measured by radio-immunoassay after pre-extraction of plasma onto Amprep minicolumns (Amersham International plc., Aylesbury, Buckinghamshire, UK) (RPN 1918) [20]. The assay has previously been described in detail [20].

Candoxatrilat (active metabolite of candoxatril). Venous blood (5 ml) was collected into plain tubes, stored on ice and spun within 2 h. Plasma was stored at -20°C and candoxatrilat, the active metabolite of candoxatril, was later estimated using an assay dependent on the ability of NEP to hydrolyse 14C-hippuryl-phenylalanyl-arginine to hippuric acid and phenyllyl-arginine. The amount of radiolabelled hippuric acid is inversely related to log concentration of candoxatrilat [21].

Drugs

Candoxatril (Pfizer Central Research, Sandwich, UK). Capsules of 200 mg were provided and administered with 200 ml of water. Placebo capsules (Pfizer Central Research, Sandwich, UK) identical to the above were provided and administered in the same way. Histamine acid phosphate (Sigma Chemical Co., Poole, Dorset, UK) was made up in phosphate buffered saline. Salbutamol (Allen & Hanbury's, Greenford, UK) was made up in 2.5 mg·ml⁻¹ aliquots by the pharmacy.

Analysis

Changes in FEV₁, pulse, blood pressure, hormonal and drug levels were compared using analysis of variance, Fisher's exact test and the Scheffe F-test were used to correct for multiple comparisons. This was performed on an Apple Macintosh SE personal computer, using the Statview software package.

Results

There was no significant difference in FEV₁ and PC₂₀ values between active and placebo study days. The mean(SEM) FEV₁ at baseline and 2 h was 3.71(0.29) and 3.85(0.29) l, respectively, on the placebo day; and

Table 2. – Pulse systolic and diastolic blood pressure (BP) following placebo or oral candoxatril (200 mg) in 10 asthmatic patients

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Pulse beats·min⁻¹</th>
<th>Systolic BP mmHg</th>
<th>Diastolic BP mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Active</td>
<td>Placebo</td>
</tr>
<tr>
<td>0</td>
<td>72.9 (6.2)</td>
<td>66.3 (3.6)</td>
<td>132 (5.8)</td>
</tr>
<tr>
<td>0.5</td>
<td>64.5 (3.8)</td>
<td>63.8 (3.7)</td>
<td>134 (5.4)</td>
</tr>
<tr>
<td>1</td>
<td>63.8 (3.4)</td>
<td>64.3 (3.2)</td>
<td>137 (4.6)</td>
</tr>
<tr>
<td>1.5</td>
<td>63.4 (3.3)</td>
<td>63.1 (2.8)</td>
<td>133 (4.9)</td>
</tr>
<tr>
<td>2</td>
<td>63.1 (3.4)</td>
<td>65 (3.7)</td>
<td>133 (5.0)</td>
</tr>
<tr>
<td>2.5</td>
<td>68.5 (3.5)</td>
<td>72.8 (3.6)</td>
<td>131 (5.0)</td>
</tr>
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<td>68.0 (3.2)</td>
<td>70.6 (2.7)</td>
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</tr>
<tr>
<td>3.5</td>
<td>67.3 (3.4)</td>
<td>73.0 (3.9)</td>
<td>135 (5.1)</td>
</tr>
</tbody>
</table>

Data are presented as mean, and SEM in parenthesis. *: p<0.05.
3.89(0.27) and 4.05(0.82) l, respectively, on the active day (fig. 1). The geometric mean (range) PC_{20} was 1.17(0.25–25.8) and 0.93(0.13–32) mg·ml⁻¹ on the placebo and active day, respectively, (fig. 2). There were no measured changes in pulse or blood pressure (BP) at 2 h, although a small significant fall in systolic BP was noted at 1 and 1.5 h on the treatment day, when compared with placebo (table 2).

Plasma drug levels were maximally elevated between 2 and 3 h (mean(SEM) plasma candoxatrilat at 1 h 161(53), 2 h 846(136), 3 h 896(68), and 4 h 62(60) ng·ml⁻¹) (fig. 3).

ANP levels rose significantly on the active day from a mean(SEM) baseline value of 12.1(2.1) pg·ml⁻¹ to 29.1 (4.8) pg·ml⁻¹ at 2 h, (p<0.005); but did not change on the placebo day, baseline 11.5 (2) pg·ml⁻¹, 2 h level 10.8(1.9) pg·ml⁻¹ (fig. 3).

Cyclic GMP levels rose on the active day from mean (SEM) baseline value of 0.69(0.28) pmol·l⁻¹ to be significantly elevated at 1.40(0.75) pmol·l⁻¹ at 2 h, and to be further elevated at 2.57(0.94) pmol·l⁻¹ at 4 h. On the placebo day there was no change with time (fig. 3).

**Discussion**

Our results demonstrate that the acute administration of the oral NEP inhibitor, candoxatril, does not alter bronchomotor tone or bronchial reactivity to histamine in stable asthmatic patients. Candoxatril is an orally active indanyl ester prodrug of candoxatrilat [15], which is a potent specific inhibitor of neutral endopeptidase [21, 22]. The oral administration of candoxatril has been shown to elevate circulating ANP levels in normal [15] and mild hypertensive patients [16]. Given by oral administration, peak plasma candoxatrilat levels are achieved at around 1.5 h, with peak ANP levels at 2–3 h, although they remain elevated at 4 h [16]. The oral administration of candoxatril (200 mg) is also associated with a significant natriuresis and diuresis [15, 16]; higher doses are not associated with higher plasma ANP levels or increased natriuresis or diuresis. NEP is ubiquitous and is plentiful in the lung, particularly in the airway [14]. The pulmonary uptake of ANP is approximately 20% [23], but it is not clear whether this is due to binding to clearance receptors or degradation by NEP within the lung. The exact contribution of lung NEP in determining circulating levels has not been quantified; however, the circulating levels of ANP doubtless reflect a general inhibition of NEP within the body. The systemic elevation of ANP does imply a significant NEP inhibitory effect and, as we have shown that NEP is important in regulating the *in vitro* effects of exogenous ANP on the airway, it is possible that inhibition of NEP within the lung may add to this and facilitate local ANP effects. Overall, we believe it is unlikely, therefore, that the absence of an effect of candoxatril on airway function was due to a failure to inhibit NEP, to the administered dose of the drug being too low, or to inappropriate timing of the bronchial challenge after dosing.

The absence of a bronchodilator response may reflect the fact that the patients had well-controlled asthma and had baseline FEV₁ values close to their predicted best. However, the plasma ANP and cGMP levels achieved were much lower than those associated with bronchodilation in our previously reported infused study [4]. At
the higher rates of infused ANP associated with bronchodilation, we also witnessed mild haemodynamic changes, with a fall in systolic BP and a rise in pulse [4]. In this study, there were no haemodynamic changes observed, except for a slight reduction in systolic blood pressure at 1 and 1.5 h. It seems likely that the levels of ANP achieved by inhibiting NEP are inadequate to exert a significant bronchodilator action on the airway in asthma. However, in patients with chronic obstructive pulmonary disease, particularly with associated pulmonary hypertension, resting ANP levels are elevated [25]. In this setting, further elevation of circulating ANP levels by the inhibition of NEP may result in pharmacological levels of ANP being achieved, allowing the manifestation of beneficial airway effects.

That ANP levels achieved are inadequate to significantly affect airway function is supported by the absence of a protective effect against histamine-induced bronchoconstriction. In concentrations lower than those required to cause bronchodilation, infused ANP has been shown to modify the airway response to inhaled histamine and to ultrasonically nebulized distilled water in asthmatics [5, 6]. However, the plasma ANP concentrations produced by these infusion studies were still higher than those achieved by the administration of candocatriol; at the time of the challenge the mean (SEM) ANP level was 29.1(4.8) after candocatriol compared with 41(4.3) pg·ml⁻¹ with infused ANP (1 pmol·kg⁻¹·min⁻¹). Inhaled ANP (1 mg) has also been shown to attenuate histamine- and methacholine-induced [7, 8] bronchoconstriction. The plasma levels associated with this dose of inhaled ANP are similar to those seen in this study (personal observation), however, it can be speculated that airway levels may be much higher when given by inhalation.

Local concentrations are likely to be the most important consideration when examining the effects of ANP on the airway, as it is probable that ANP achieves its effects \textit{in vivo} by acting directly on smooth muscle in the airway. ANP is thought to act on particulate guanylyl cyclase to cause the generation of cGMP, which is believed to be the second messenger for ANP in the cell [25]. Evidence for this includes several studies in animal models, in which a direct dose-relaxant effect of ANP in airway smooth muscle has been demonstrated, and work on isolated bovine trachea suggesting that this mechanism is likely to be mediated by cGMP [26, 27]. ANP receptors have also been demonstrated in the rat lung, and these are located in the bronchial and bronchiolar muscle [28], although there are no studies, so far, which have sought ANP receptors on the human airway. Further evidence for a direct action is the observation that ANP confers protection against methacholine-induced contraction in isolated human and bovine bronchial tissue [10].

Whilst no beneficial effects were noted with the administration of candocatriol, neither were any side-effects observed or reported. Likewise, there was no increase in airway responsiveness to histamine. NEP cleaves a variety of active peptides in addition to ANP, including encephalins, tachykinins (such as neurokinin A and substance P), bradykinin, and chemotactic peptide [11]. A number of substances have bronchoconstrictor properties, including tachykinins, which may be involved in neurogenic inflammation and the pathogenesis of asthma [2, 29]. It might be predicted, therefore, that inhibition of NEP would increase bronchial reactivity. \textit{In vitro} work on human tissue has demonstrated that inhibition of NEP potentiates substance P and capsaicin-induced bronchial smooth muscle contraction [30]. Thorphan, an NEP inhibitor, has been given by inhalation to normal subjects and asthmatic patients; no effect on airway tone was noted, and there was no effect on a subsequent bronchial challenge by methacholine [31, 32]. However, there was an enhanced bronchoconstrictor response to the neuropeptide, neurokinin A [31, 32]. One other study looked at responsiveness to the inflammatory mediator leukotriene D₄ in normal subjects, and found that preinhalation with thorphan enhances its effect [33]. In a study of an orally-administered NEP inhibitor, acetorphan, in a dose sufficient to enhance skin responses to substance P, no effect on airway function or responsiveness to inhaled metabisulphide, which is thought to act in part by the release of sensory neuropeptides, was noted in mildly asthmatic subjects [34]. The role of NEP in the lung is unclear, but is a balance between the potential beneficial effects of inhibiting ANP metabolism against the deleterious effects of inhibiting the breakdown of other agents, such as tachykinins. No measure of substance P or neurokinin A has been made, and we cannot exclude that a challenge with these tachykinins might have resulted in bronchoconstriction. Nevertheless, our results suggest that in mild stable asthmatics the acute systemic inhibition of NEP does not alter the bronchial response to histamine.

Overall, the effect of the acute inhibition of NEP on airway tone and nonspecific reactivity appears to be neutral in these mild stable asthmatics. It is reassuring that no deleterious effects on airway function have been observed, particularly if NEP inhibitors prove useful in the treatment of other conditions, such as cardiac failure. A cautionary note is required, however, as this study has only examined the acute effects of inhibiting NEP in patients with mild stable asthma, and does not address changes in NEP function or levels which may occur in patients with more severe asthma. Our findings would suggest that the systemic inhibition of NEP will not allow the beneficial effects of ANP on the airway to be harnessed in patients with asthma. One possible intervention that could be envisaged, however, is the administration of an NEP inhibitor concomitantly with ANP by inhalation. Here it could be speculated that the local inhibition of airway NEP would promote the effects of ANP. Alternatively, if the guanylyl cyclase pathway acted upon by ANP is to be exploited therapeutically in asthma, it seems likely that ANP analogues stable to NEP would hold more promise.

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References


