

by Wang therefore rapidly gained popularity.<sup>3</sup> Although the sensitivity of this technique was initially reported by Wang as 76%,<sup>4</sup> we and others found the needle difficult to lodge into the mediastinum and this is reflected in subsequent reports of variable sensitivity in the diagnosis of lung and mediastinal malignancy.<sup>7-10</sup> Since the early descriptions of the use of a flexible needle Wang has devised both an 18 gauge and 19 gauge needle. Overall, sensitivity using the Wang 18 gauge needle increased to 80% and with the 19 gauge needle to 89%.<sup>5,11</sup> Both needles provide histological material for diagnosis. It is noteworthy that in these latter two studies CT staging was employed and so the improved sensitivity may in part have been due to better selection of the sample sites.

The presence of a cytopathologist in theatre has had a major impact on the sensitivity of TTNA in our institution with an improvement from 18.8% in an earlier study<sup>6</sup> to 81%. The mean number of aspirates per procedure has increased but without complication. This study has shown that the sensitivity of TTNA has increased still further when a preoperative CT scan is available to guide the operator to the best site for sampling. TTNA using the flexible needle is safe with only a few reports of complications and rare false positive aspirates.<sup>12-14</sup>

We have experienced no major complications with the rigid needle in this institution.

We thank the physicians and bronchoscopy staff who allowed us to use their patient records and Miss M McKinlay for typing the manuscript.

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*Thorax* 1996;51:199-201

## Angiotensin converting enzyme (ACE) inhibitor-induced cough and substance P

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### Abstract

**Background** - Angiotensin converting enzyme (ACE) inhibitors cause coughing in 5-10% of patients, but the exact mechanisms of this effect are still unclear. In the airways ACE degrades substance P so the cough mechanism may be related to this peptide.

**Methods** - Nine patients who developed a cough and five patients who did not develop a cough when taking the ACE inhibitor enalapril (2.5 or 5.0 mg/day) for hypertension were enrolled in the study. No subjects had respiratory disease and the respiratory function of all subjects was normal. One month after stopping enalapril, inhalation of hypertonic saline (4%) was performed using an ultrasonic nebuliser for 15-30 minutes to induce sputum. The concentration of substance P in the sputum sample was measured by

radioimmunoassay. In four of the nine cases with a cough enalapril was given again for 1-2 weeks and the concentration of substance P in the induced sputum was again measured.

**Results** - One month after stopping enalapril the mean (SE) concentration of substance P in the sputum of the group with a cough was 16.6 (3.0) fmol/ml, significantly higher than that in the subjects without a cough (0.9 (0.5) fmol/ml). All four subjects in the group with a cough who were given a repeat dose of enalapril developed a cough again, but the concentrations of substance P in the induced sputum while taking enalapril (17.9 (3.2) fmol/ml) were similar to the values whilst off enalapril (20.0 (2.5) fmol/ml).

**Conclusions** - The mechanisms of ACE inhibitor-induced coughing may involve substance P mediated airway priming.

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Received 9 January 1995  
Returned to authors 23 March 1995  
Revised version received 8 September 1995  
Accepted for publication 9 October 1995

**However, the final triggering of the ACE inhibitor-induced coughing is unlikely to be due to this peptide.**

(*Thorax* 1996;51:199–201)

Keywords: neurogenic inflammation, substance P, ACE inhibitor.

Angiotensin converting enzyme (ACE) inhibitors are known to cause coughing in 5–10% of patients,<sup>1</sup> but the precise mechanism is still unclear. In the airways ACE degrades bradykinin and tachykinins such as substance P.<sup>2,3</sup> Substance P has been reported to have a variety of effects on the airway including inducing cough,<sup>2,4</sup> so it is possible that ACE inhibitor-induced coughing is due to decreased metabolism of substance P. To test this hypothesis we have measured the concentration of substance P in induced sputum after inhalation of hypertonic saline in patients with and without ACE inhibitor-induced cough.

### Methods

The subjects were chosen from patients who had taken the ACE inhibitor enalapril (2.5 or 5.0 mg/day). Written informed consent for the protocol, which was reviewed and approved by the Tohoku University Committee on Clinical Investigation, was obtained from each patient. Patients were divided into two groups according to whether they coughed while taking enalapril or not. No subjects had respiratory disease and the respiratory function, measured as vital capacity (VC), forced expiratory volume in one second (FEV<sub>1</sub>), and arterial blood gas analysis, of all subjects was within normal limits. VC and FEV<sub>1</sub> were measured using a dry rolling seal spirometer (OST 80A, Chest Co, Tokyo, Japan) and arterial oxygen and carbon dioxide tensions (P<sub>a</sub>O<sub>2</sub> and P<sub>a</sub>CO<sub>2</sub>) and pH were determined with a pH/blood gas analyser (Model 1312; Instrumentation Laboratory Co, Lexington, Massachusetts, USA).

Each subject had stopped taking enalapril at least one month before the sputum induction. In four of the cases with a cough enalapril was given again for 1–2 weeks and the concentration of substance P in the induced sputum was again measured.

Hypertonic saline (4%) inhalation was performed with an ultrasonic nebuliser<sup>5</sup> for 15–30 minutes until a sputum volume of about 1 ml was collected. Because the samples contained saliva, we eliminated this contamination by visual inspection and inverted microscope examination. The sputum samples were immediately mixed with an inhibitor solution (2 × 10<sup>-5</sup> M neutral endopeptidase inhibitor phosphoramidon, 2 × 10<sup>-5</sup> M kininase II inhibitor captopril, 500 kU/ml serine protease inhibitor aprotinin, and 1.2 mg/ml EDTA) to avoid degradation of substance P and stored at -70°C until assay.<sup>6</sup>

The sputum samples were diluted with acetic acid (4%, pH 4.0 adjusted by ammonium) to a final volume of 50 ml, homogenised using Polytron PT-10 (PT-35, Kinematica AG, Lit-

tau, Switzerland), and centrifuged at 40 000 *g* for 30 minutes. The obtained supernatant was loaded on reversed phase C18 cartridges (Sep-Pak C18, Millipore Co, Milford, Massachusetts, USA). After washing with 20 ml 4% acetic acid (pH 4.0) and 20 ml distilled water, substance P was eluted with 2 ml 80% acetonitrile in 0.1% trifluoroacetic acid. Elutes were concentrated by spin vacuum evaporation, lyophilised, and dissolved with 0.15 ml assay buffer (50 nM phosphate buffer, pH 7.2, containing 3.7 g/ml EDTA and 0.5% bovine serum albumin). A total of 0.1 ml of the dissolved preparation was subjected to further radioimmunoassay for substance P. Radioimmunoassay for substance P was performed using <sup>125</sup>I-labelled substance P (Amersham International, Amersham, UK) and anti-substance P rabbit serum (Amersham International). 0.1 ml samples were mixed with 0.5 ml assay buffer, 0.1 ml antiserum, and 0.1 ml <sup>125</sup>I-substance P and stored at 4°C for 24 hours. A total of 0.2 ml dextran/charcoal suspension (0.2% dextran and 2% activated charcoal in assay buffer) was added to the reaction mixture and centrifuged at 2000 *g* for 10 minutes. The radioactivity of the supernatant was measured by a gamma counter (Model 5420, Packard Instrument Co, Meriden, Connecticut, USA). The concentration of substance P was calculated in terms of ml of induced sputum. In this system the sensitivity of immunoassayable substance P in saline was 1–60 fmol/ml. Furthermore, to determine how much substance P in the sputum was measurable we added substance P (30 fmol) to the sputum and measured the immunoreactivity of the peptide. In such cases 99.3 (4.3)% of immunoassayable substance P was detected.<sup>6</sup>

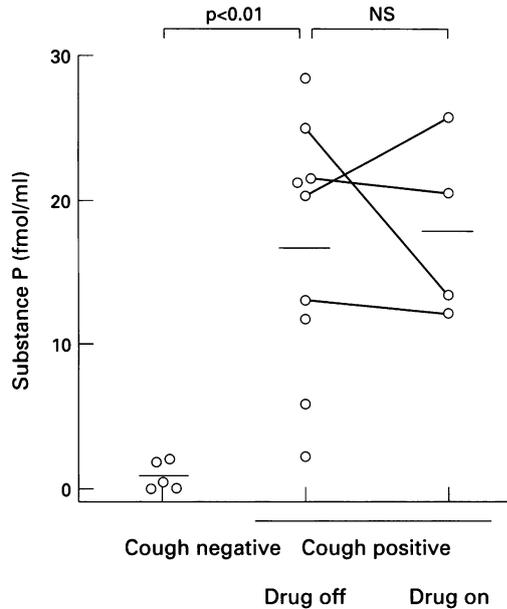
### DATA ANALYSIS

Data are expressed as mean (SE). Comparisons of the concentrations of substance P in induced sputum between the two groups were made with the Mann-Whitney U test (two tailed). A *p* value of <0.05 was considered significant.

### Results

The cough positive group consisted of nine subjects (three men) of mean age 60.6 years (range 46–74), of whom one was a smoker and two were ex-smokers. The cough negative group consisted of five subjects (two men) of mean age 53.2 years (range 37–72), three of whom were smokers.

Inhalation of hypertonic saline caused sputum production (approximately 1 ml) in each case. There was no significant difference in the mean volume of sputum induced in the two groups (0.9 (0.3) ml for the cough positive group and 0.8 (0.1) ml for the cough negative group). The percentage of squamous cells in the sputum was less than 10% in all subjects, indicating that the sample of sputum was primarily from the tracheobronchial tree.



Concentration of substance P in the induced sputum of patients with coughing (cough positive) and those without coughing while taking enalapril (cough negative). Drug on and off indicate taking and stopping enalapril, respectively. Lines indicate data from the same subject. Bars represent the mean values.

The concentrations of substance P in the sputum samples are shown in the figure. In the cough positive group the substance P concentrations were significantly higher (16.6 (3.0) fmol/ml) than those of the cough negative group (0.9 (0.5) fmol/ml) ( $p < 0.01$ ). All four subjects in the cough positive group who were given a repeat dose of enalapril developed a cough again, but the concentrations of substance P in the induced sputum while taking enalapril (17.9 (3.2) fmol/ml) were not significantly different from the values while off the drug (20.0 (2.5) fmol/ml).

### Discussion

In this study we have shown that the concentration of substance P in sputum induced by hypertonic saline is significantly higher in patients with ACE inhibitor-induced coughing than in those without coughing one month after stopping enalapril. In the airways substance P is localised in sensory C fibres.<sup>2</sup> Hypertonic saline inhalation challenge releases tachykinin from airway sensory nerves.<sup>7</sup> The increased concentration of substance P in the induced sputum of the cough positive group may therefore be explained by the increased release of substance P from sensory nerves or decreased breakdown of substance P in the airways. Substance P has various actions on the airways including coughing and, in guinea pigs, even a low concentration of substance P can cause coughing.<sup>4</sup> Taken together, substance P may be involved in the pathogenesis of ACE inhibitor-induced coughing.

Because ACE inhibitors were stopped one month before the measurement of substance P

concentrations, it is unlikely that the inhibition of substance P degradation by the ACE inhibitor is responsible for the increased concentrations of substance P in the induced sputum. It is therefore likely that increased production or decreased degradation of substance P already existed before ACE inhibition. We have recently reported that substance P levels in the induced sputum of patients with asthma and chronic bronchitis are higher than in the sputum of healthy subjects, and that substance P-mediated inflammatory mechanisms may contribute to the airways obstruction.<sup>6</sup> In the present study all subjects were free from respiratory symptoms and the pulmonary function of all subjects was normal, but the concentration of substance P in the patients with ACE inhibitor-induced coughing was similar to that in patients with asthma and chronic bronchitis. The reason for the difference between our previous study and the results reported here is unknown, but it is possible that there is a subgroup in whom the concentration of substance P in the airways is high, and may play a part in causing coughing while taking ACE inhibitors in normal subjects free from airway diseases. The percentage of the subgroup seems low, because ACE inhibitors cause coughing in only 5–10% of patients taking them. The recent report in which we showed a low concentration of substance P in the airways of subjects free from airways disease<sup>6</sup> had only nine normal subjects so we might have missed the existence of such a subgroup.

In conclusion, the mechanisms of ACE inhibitor-induced coughing may involve substance P-mediated airway priming, but the final triggering of ACE inhibitor-induced coughing is unlikely to be due to this peptide. Other peptides such as bradykinin which are also degraded by ACE may be important in triggering coughing. Newly developed substance P receptor antagonists<sup>8</sup> used for patients with coughing induced by ACE inhibitors may be useful for confirming this hypothesis.

We are indebted to Mr Brent Bell for reading this manuscript.

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