Increased Bronchial Reactivity and Potentiated Skin Responses in Hypertensive Subjects Suffering from Coughs during ACE-inhibitor Therapy

Björn R. Lindgren, M.D., Ph.D.; Ulf Rosenqvist, M.D.; Tommy Ekström, M.D.; Reidar Gröneberg, M.D., Ph.D., F.C.C.P.;† Bengt E. Karlberg, M.D., Ph.D.;‡ and Rolf G. G. Andersson, Ph.D.§

The aim of this study was to investigate whether ACE-inhibitors could influence bronchial reactivity and interfere with inflammatory skin responses. Ten hypertensive subjects, who had reacted with coughs during ACE-inhibitor therapy, were treated in a double-blind crossover fashion for two weeks with enalapril and with placebo. Enalapril reduced the PC_{10} value for histamine and augmented the dermal response. Circulating eosinophilic leukocyte level in venous blood dropped markedly after the histamine bronchoprovocation performed during enalapril treatment. Plasma substance P was reduced after histamine provocation performed during placebo treatment, whereas this reduction was abolished by enalapril. In this study, we have demonstrated ACE-inhibitor induction of moderately increased bronchial reactivity in subjects with suspected ACE-inhibitor elicited coughs. It is suggested that coughing during ACE-inhibitor therapy is due to an increased inflammatory state in the airways. (Chest 1989; 95:1225-30)

PC_{10} = histamine concentration causing a 20 percent reduction of FEV_{1}; PRA = plasma renin activity

After the discovery and isolation of teprotide, the first known ACE inhibitor, from the venom of the Brazilian pit viper Bothrops jararaca, synthesized ACE inhibitors have become widely used in the treatment of hypertension and congestive heart failure. In addition to inhibiting the conversion of angiotensin I to II, ACE inhibitors have been demonstrated to decrease the metabolism of bradykinin1,2 and substance P,3,4 which are important proinflammatory mediators. Adverse skin and mucosal reactions are associated with ACE-inhibitor treatment.5,6 Lately, interest has been focused on ACE inhibitor-induced coughs which seem to occur more frequently than was previously believed, possibly because of difficulties both patients and doctors have in relating this symptom to the antihypertensive therapy. Several studies have reported suspected ACE inhibitor-induced coughs in about 10 to 30 percent of studied subjects.6-10 It has been suggested that cyclooxygenase products might be involved in the pathogenesis of this cough, since the cyclooxygenase inhibitor sulindac relieved symptoms in some patients17 and since ACE inhibition is known to interfere with the production of prostaglandins.18 Capsaicin is a naturally occurring substance derived from plants of the Solanaceae family and known as the pungent of chilies. Its mode of action is not completely understood, but capsaicin causes an initial release as well as a depletion of neuropeptides, eg, substance P, from primary sensory neurons.19,20 The capsaicin-induced cough reflex has been demonstrated to be augmented following ACE-inhibitor treatment.11,21 Bronchial reactivity to histamine and bradykinin was not changed in six subjects with mild asthma given one dose of ramipril.22 A good correlation exists between bronchial and dermal inflammatory responses,23 and ACE inhibitor-induced adverse reactions in the airways and the skin occasionally have been observed to be accompanied by eosinophilia.13,24,25 Infiltration of inflammatory cells, eg, eosinophils, into allergen-treated test sites in sensitized guinea pigs was increased by ACE inhibition as well as the wheal and flare reactions evoked by allergen, capsaicin, and bradykinin.26,27

The aim of the present study was to investigate whether ACE inhibition could influence bronchial reactivity and anti-human IgE-evoked skin responses.
in subjects with coughs previously strongly suspected of being ACE inhibitor-induced. The study was approved by the ethical committee of Linköping University Hospital.

**MATERIALS AND METHODS**

**Subjects and Treatment**

Study subjects were ten hypertensive patients, three men and seven women (Table 1), who had previously reacted with persistent coughs during ACE-inhibitor therapy. The subjects were skin tested with anti-human IgE and histamine bronchoprovocation, at the end of two-week-long treatment periods, with enalapril (10 mg twice a day) and placebo. Drugs were administered in a randomized, double blind cross-over manner and given throughout the four-week-long study. All antihypertensive medication was suspended during a three-week run-in period. One subject (No. 5) had stable bronchial asthma and continued with regular inhalations of corticosteroids twice daily throughout the study.

**Intradermal Skin Testing**

Intradermal skin testing was performed between days 13 and 27, and the inflammatory response was followed for 24 h. At the beginning of each test, 15 µL of diluted (1/200 vol/vol ratio) heterologous whole anti-human IgE, raised in rabbits against a mixture of isolated Fc fragments from the epsilon chain from a human IgE myeloma protein, was injected intradermally. This was done by means of an Agla micrometer syringe outfit (Wellcome Reagents Ltd, Beckenham, England) in two skin sites in the volar area of the forearms. One injection was given in the distal half of one arm, and the other in the proximal half of the other arm. Two weeks later tests were performed on the half of the forearm that had not previously been used in order to avoid the problem of the skin being in a refractory phase. Two perpendicular diameters of the roughly circular flare/erythema reaction areas were measured 0.25, 0.5, 1, 3, 6, 12 and 24 h after the intradermal injections of anti-IgE. The mean diameter area was calculated, assuming a circular shape, according to the formula:

\[
\text{mean diameter} = \pi \times \left( \frac{\text{area}}{\pi} \right)
\]

In order to find and exclude subjects sensitized to rabbit IgG, the patients were also tested intradermally with normal rabbit IgG.

**Histamine Bronchoprovocation**

Histamine provocations were started on days 14 and 28. A modified version of the two-minute tidal breathing method of Cockcroft and co-workers was used. Physiologic saline solution and then doubled concentrations of histamine were administered via a nebulizer (Aiolos System, Karlstad, Sweden) for 2 min (0.7 ml/min, average particle size 0.4 µm). The bronchial response was measured by FEV, 2 min after each inhalation, and the procedure was continued until a fall in baseline FEV, of 20 percent or more was reached. Results were expressed as PC_{20}. This was obtained by linear interpolation of the last two points on the log dose-response curve.

**Blood Tests**

The ACE activity in serum was determined by a colorimetric assay (Kit, Buhlman Laboratories, Switzerland)\(^{19}\) and, like PRA, analyzed prior to the study and at the end of placebo and enalapril treatment periods. Plasma substance P (Neurokemlab, Lund, Sweden)\(^{20}\) and circulating eosinophils were measured immediately prior to and after the histamine bronchoprovocation. Eosinophils in venous blood were also estimated prior to, six and 24 h after intradermal anti-IgE injections.

**Drugs**

Gelatin capsules (No. 0, E2010, Apoteksbolaget, Sweden) containing enalapril maleate, 10 mg mixed with lactose, and placebo capsules were prepared and randomized.

**Statistical Analyses**

Student’s paired t test was used to evaluate differences between the effects of enalapril and the placebo on ACE, PRA, blood pressure, PC_{20}, eosinophils, and substance P. Analysis of variance was used to study differences between dermal flare/erythema reactions during enalapril and placebo treatment periods.

**RESULTS**

**ACE and PRA**

During enalapril treatment, ACE was effectively inhibited (difference from placebo treatment, -0.74 ± 0.06 µkat/L; p < 0.01) and PRA increased (difference from placebo, +0.88 ± 0.1 µkat/L; p < 0.05) (Fig 1).

**Cardiovascular Parameters**

Diastolic blood pressure was significantly decreased during enalapril treatment (difference, 5.6 mm Hg; p < 0.05) but not during treatment with the placebo.
Systolic blood pressure and pulse frequency were not significantly changed (Fig 2).

**Skin Responses**

The flare/erythema response following intradermal injection of anti-IgE was augmented (ANOVA; p = <0.05) by enalapril, and the inflammatory reaction during enalapril, as well as during placebo, treatment showed a dual response with a second maximum 12 h after testing. The late phase reaction was augmented especially prominently by enalapril at 12 h (p<0.01) (Fig 3). None of our patients reacted to intradermally injected normal rabbit IgG.

**Lung Function and Bronchoprovocation**

Lung function values and the incidence of coughs, determined at the start of the study, were not changed by the ACE inhibitor or the placebo treatment when checked prior to the bronchoprovocations performed at the end of each treatment period. However, compared with the placebo, enalapril increased bronchial reactivity, and the mean PC_{20} value was decreased from 12.7 ± 2.5 to 8.8 ± 1.7 (± SEM) mg histamine (30 percent; p<0.05). The asthmatic subject increased her bronchial reactivity to histamine in a way similar to the other patients, but no extra need for β-adrenoceptor agonist inhalations was noticed during the enalapril treatment period.

**Eosinophils**

The number of circulating eosinophilic leukocytes in venous blood was reduced 6 h after the intradermal injections of anti-IgE. The drop tended to be more pronounced, although not significantly so, during enalapril treatment (33 percent; p<0.05) than during placebo treatment (25 percent; p<0.05).

The number of eosinophils in venous blood also
decreased after histamine bronchoprovocations, and the drop was more pronounced during enalapril treatment (36 percent; \( p<0.01 \)) than during placebo treatment (12 percent = NS) (Fig 4). However, the difference (\( -65 \times 10^6/L \)) between placebo and enalapril treatment was not significant (\( p<0.06 \)).

**Substance P**

Plasma substance P concentrations before and after bronchoprovocations were within the normal reference range (\(<4.0\) pmol/L). However, interesting trends were observed. Thus, the values obtained prior to the histamine bronchoprovocations performed during placebo treatment were higher than after the provocations (\( p<0.05 \)). This reduction was not observed following the provocations performed during enalapril treatment. Furthermore, the plasma substance P concentrations prior to the bronchoprovocations were lower during enalapril treatment than during placebo treatment (\( p<0.01 \)) (Fig 5).

**DISCUSSION**

Treatment with enalapril for nearly two weeks did not result in any increase in the incidence of coughs compared with placebo treatment at the same time. This was, perhaps, what could be expected since most of our patients anamnestically developed suspected ACE inhibitor-induced coughs more than two weeks after beginning ACE-inhibitor therapy. However, in our patients, enalapril treatment increased bronchial reactivity to histamine. It is difficult to judge if this moderate increase of bronchial reactivity is a truly physiologic response or not but it supports the results of Bucknall et al.,

**determine whether increased histamine bronchoprovocation sensitivity is a phenomenon peculiar to patients who develop a cough on ACE inhibition or is a general phenomenon among patients treated with ACE inhibitors.**

The magnitude of the change in reactivity in the asthmatic subject in our study was modest and comparable to the change in the other patients. However, she had stable bronchial asthma and continued to use inhaled steroids throughout the study, which could have, at least in part, masked the proinflammatory properties of the ACE-inhibitor. Others have reported an almost immediate deterioration of lung function after ACE-inhibitor treatment was initiated in asthmatic subjects. Enalapril, 10 mg administered twice daily, effectively inhibits dipeptidylcarboxypeptidase (ACE) throughout a whole day. This drug effect thereby fulfills the prerequisite for prolonged activity of otherwise quickly metabolized proinflammatory mediators like bradykinin and substance P. Bradykinin can stimulate afferent polymodal C-fiber endings, and via local axon reflexes, probably cause the release of neuropeptides like substance P. Histamine also can activate these sensory nerves and we have recently demonstrated that MK 422 (the active parent diacid of enalapril) increased spontaneous histamine release from guinea pig skin and lung tissue and from human basophilic leukocytes. Substance P, and the presumably coreleased peptides calcitonin, gene-related peptide and neurokinin A, have direct proinflammatory effects on blood vessels and might also, via "activation" of mast cells, contribute to an augmented inflammatory response. Some products generated during this complex reaction chemotactically activate inflammatory cells like eosinophils and neutrophils. We could also demonstrate a more pronounced drop in the number of eosinophils in venous blood after the provocations performed during ACE-inhibitor treatment. Our results, demonstrating an increased influx of inflammatory cells (eg, neutrophils and eosinophils) into inflammatory dermal test sites in ACE inhibitor-treated guinea pigs, suggest that the reduction in the number of circulating eosinophils, observed after the provocations in our patients, is due to an influx of these cells into the provoked tissue. Cationic protein, derived from eosinophils, can activate the "Hageman factor" and thereafter contribute to formation of bradykinin via the "kalikrein kinin system." Bradykinin could then initiate the release of more proinflammatory neuropeptides, leading to a progressively increasing inflammatory reaction. Since bronchial reactivity is closely related to the inflammatory state in the airways, previously described mechanisms for ACE inhibitor-induced/potentiated inflammation might be speculated to account for the increased bronchial
reactivity seen in our patients during enalapril treatment.

Involvement of chemosensitive, unmyelinated sensory neurons in the cough reflex is supported by observations of inhaled capsaicin-producing coughs in guinea pig and man\textsuperscript{11,21,46} and ACE-inhibition potentiating capsaicin-induced coughs.\textsuperscript{11,21} Capsaicin selectively stimulates these C-fiber afferents, which could be visualized in the skin flare response.\textsuperscript{46} The flare/erythema response in our patients was potentiated by enalapril, which further supports a role for “neurogenic inflammation” in ACE inhibitor-induced coughs.

The possibility that ACE-inhibitors interfere with the inflammatory state in diseases where bradykinin and substance P can play a role in the pathophysiology must always be kept in mind when treating hypertensive patients with this type of drug.

ACKNOWLEDGMENTS: Professor S. Gunnar O. Johansson, Department of Clinical Immunology, Karolinska Hospital, Stockholm, kindly supplied heterologous whole anti-human IgE. Dr. Sune Lindgren, Pharmaceutical Division, University Hospital, Linköping, Sweden, prepared and randomized the enalapril maleate gelatin capsules and placebo capsules.

REFERENCES

1 Ferreira SH. A bradikinin-potentiating factor (BPF) present in the venom of Bothrops jararaca. Br J Pharmacol Chemother 1965; 24:163-69
9 Andersson RGG, Karlberg BE, Lindgren BR. Cough and other bronchial symptoms and skin disorders during treatment with ACE-inhibitors (Swedish). Lakartidningen, 1987; 84:183-84
12 Hood S, Nicholls MG, Gilchrist NL. Cough with angiotensin converting enzyme inhibitors. NZ Med J 1987; 100:6-7
17 Nicholls MG, Gilchrist NL. Sulindac and cough induced by converting enzyme inhibitors. Lancet 1987; 1:872
23 Boulet LP, Roberts RS, Dolovich J, Hargrave FE. Prediction of late asthmic responses to inhaled allergen. Clin Allergy 1984; 14:379-85
Plan to Attend
55th Annual Scientific Assembly—
XVI World Congress on Diseases of the Chest

BOSTON 1989

ACCP

Boston • October 30-November 3, 1989

1230

Increased Bronchial Reactivity and Skin Responses in Hypertension (Lindgren et al.)