Endothelin-1 levels in induced sputum samples from asthmatic and normal subjects

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Abstract

***Background* – Endothelin-1 (ET-1) is a potent bronchoconstrictor which may have a role in the pathogenesis of asthma. The levels of ET-1 in saliva, induced sputum, and plasma from asthmatic and non-asthmatic subjects were compared.**

***Methods* – Sputum induction was performed on 28 asthmatic subjects and nine normal volunteers. ET-1 levels were measured in plasma, saliva, and sputum samples and reversed phase high performance liquid chromatography (RP-HPLC) was performed on saliva and sputum samples.**

***Results* – ET-1 was present in the following order of concentration in both normal and asthmatic subjects: saliva > sputum > plasma (saliva, median 30.1 and 23.9 pg/ml, respectively; sputum, median 15.5 and 11.2 pg/ml; plasma, median 3.1 and 3.6 pg/ml). There were no differences between asthmatic and normal subjects in the levels of ET-1 in each fluid. The levels of ET-1 in asthmatic subjects were not influenced by whether or not they were taking inhaled steroids. RP-HPLC of sputum and saliva confirmed the presence of ET-1 in these fluids.**

***Conclusions* – Levels of ET-1 can be measured in saliva and sputum obtained by sputum induction in asthmatic and healthy subjects and, although no difference was found in basal levels of ET-1 in sputum, saliva and plasma between normal subjects and asthmatics without bronchoconstriction, it is apparent that ET-1 is produced or released locally within the respiratory tract in concentrations higher than those in plasma.**

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It is postulated that endothelins (ET) may have a role in the pathogenesis of asthma by various mechanisms, including direct bronchoconstriction, since ET-1 is one of the most potent bronchoconstrictor peptides yet isolated. ET-1 is produced by human bronchial epithelial cells, and the bronchial epithelial cells of asthmatic patients, but not of normal subjects, show increased expression of endothelin.[1] Elevated levels of endothelin have been reported in bronchoalveolar lavage fluid of symptomatic and non-steroid treated asthmatic patients.[2]

Sputum induction has been developed as an alternative technique for sampling of airway secretions which, by contrast with bronchoscopy, is non-invasive, generally well tolerated, and can be repeated after a short interval if required. The objectives of the study were to establish whether ET-1 could be measured in fluid produced by sputum induction, to compare the levels of ET-1 in saliva, sputum and plasma, and to compare basal levels of ET-1 in each fluid between asthmatic and non-asthmatic subjects.

Methods

SUBJECTS AND SPUTUM INDUCTION

Twenty eight stable asthmatics and nine normal subjects who were non-smokers were studied. Asthma was defined by clinical symptoms, response to inhaled β_2 agonist, and a methacholine PC_{20} of <8 mg/ml. Normal subjects were asymptomatic and had a methacholine PC_{20} of >16 mg/ml with normal baseline spirometric measurements. The study was approved by the West Ethics Committee, West Glasgow Hospitals University NHS Trust, and each subject gave written informed consent.

Sputum induction was performed using a modification of the method described by Pin *et al*.[3] Briefly, after salbutamol 200 μg was administered, saliva was collected over the next five minutes, followed by blood sampling and sputum induction using hypertonic (3%) saline administered via an ultrasonic nebuliser (Schuco International Ltd, London, UK) over a period of 20 minutes. Samples were collected in sterile containers and transferred to the laboratory on ice, with laboratory staff not informed of clinical details. ET-1 in plasma samples did not alter as a result of sputum induction per se (subgroup n = 9, data not shown).

LABORATORY PROCESSING

Sputum plugs were selected (minimum 50 mg) to minimise salivary contamination and were split into two, half of which were treated with fresh dithiothreitol (DTT) (Sigma UK Ltd) in a balanced salt solution and used for cell counts and assay of eosinophilic cationic protein (ECP), and the other half were centrifuged at 13 000 rpm for 15 minutes without prior treatment with DTT for ET-1 assay as we found that DTT altered the standard curve for the ET-1 assay. Differential cell counts (Giemsa stained) were expressed after exclusion of squamous epithelial cells which were taken

to represent salivary contamination. Reversed phase high performance liquid chromatography (RP-HPLC) was carried out on saliva and sputum samples. During the gradient elution 10 × 1 ml fractions were collected between 15 and 25 minutes (synthetic ET-1 elution point was 21.1 minutes). Endothelin was assayed using a radioimmunoassay (RIA) (Nichols Institute Diagnostics Ltd, San Juan Capistrano, California, USA) with a lower limit of detection of 1.6 pg/ml in plasma following pre-extraction using C-18 columns (Sep-pak, Waters Ltd Watford, UK). Recovery of ET-1 from sputum was 88%. ECP was assayed using a radioimmunoassay with a lower limit of detection of 2 μg/l in plasma (Pharmacia UK Ltd, Milton Keynes, UK).

STATISTICAL ANALYSIS

Non-parametric statistics were used to compare cell counts, ECP, and ET-1 values. Significance was accepted at the 95% level. Power calculations suggest a power of >85% to detect a difference in sputum levels of ET-1 of ± 1 SD.

Results

The asthmatic group was older (mean (SD) age 41.4 (10) years versus 31.8 (8.8) years), and had lower forced expiratory volume in one second (FEV_1) values than normal subjects, both as absolute values (2.99 (0.9) l versus 3.93 (1.08) l) and percentage predicted (84.0 (14)% versus 99.2 (11.4)%). The mean daily dose of inhaled corticosteroid was 400 μg beclomethasone or equivalent in the asthmatic group. The levels of eosinophils in the sputum were higher in the asthmatic group (median 3%) than in the normal subjects (median 0%), and no differences were seen in sputum volumes, total cell counts, or other cells. A comparison of median (IQR) plasma, saliva and sputum levels of ET-1 between each group showed no significant differences between asthmatic and normal subjects for each fluid (asthmatics, saliva 23.8 (14.4–33.7) pg/ml; sputum 11.2 (9.4–18.9) pg/ml; plasma 3.6 (2.8–4.5) pg/ml: normal subjects, saliva 30.1 (20.1–43.4) pg/ml; sputum 15.5 (10.0–21.4) pg/ml; plasma 3.1 (1.7–4.8) pg/ml). Pairwise analysis of asthmatic subgroups revealed no differences between those taking inhaled steroids (n = 16) and those taking inhaled β_2 agonists alone (n = 12). There were significant differences within each patient group in the levels of ET-1 in the order saliva > sputum > plasma (fig 1). No significant direct correlation was found between plasma or sputum levels of ET-1 and the FEV_1 or methacholine PC_{20} in the asthmatic patients. RP-HPLC of saliva and sputum samples revealed a single peak for both saliva and sputum corresponding to the elution point of the pure ET-1 standard. In both asthmatic and normal subjects the median (IQR) sputum level of ECP (129.0 (100–340) μg/l and 340.0 (134–710) μg/l, respectively) was higher than the serum level of ECP, with serum ECP levels being higher in asthmatic subjects than in normal controls (9.0 (3–11) μg/l versus 1.0 (0–7) μg/l). Saliva levels of ECP were assayed in a subgroup of seven subjects drawn from each of the groups at random to compare with sputum. In this group the median (IQR) levels of ECP in the saliva (10.0 (5–69) μg/l) were lower than sputum levels of ECP (210 (26–460) μg/l), suggesting that sputum levels of ET-1 do not represent salivary contamination.

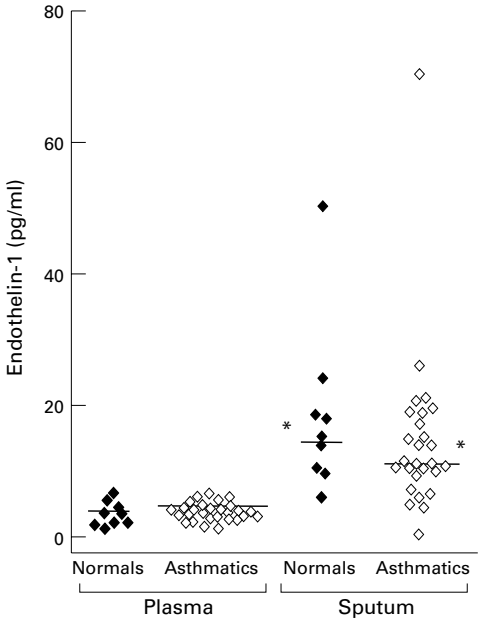

*Figure 1 Plasma and sputum levels of endothelin-1 in normal (n = 9) and asthmatic (n = 28) subjects. Horizontal lines indicate median values. *p<0.05 sputum versus plasma levels.*

Discussion

This study shows that it is possible to measure ET-1 by RIA and to confirm its identity by RP-HPLC in sputum obtained by sputum induction, and that saliva and sputum levels of ET-1 are higher than plasma ET-1 levels in both normal and asthmatic subjects. Our findings for ECP would suggest that the levels of mediators present in sputum do not simply represent contamination from saliva. The finding that ET-1 levels are higher in saliva than in sputum is unexpected and its role in saliva is unknown. ET-1 has been measured in rat parotid gland and human saliva,[4] although at lower levels than we observed. The finding of raised saliva levels of ET-1 also suggest that mediators which are being sought in sputum should also be assessed in saliva to avoid misleading results. Allowing, as far as can be determined, for dilution of bronchoalveolar lavage (BAL) fluid samples, our finding of sputum levels of ET-1 of 10–15 pg/ml accords reasonably with levels in BAL fluid found by other groups.[2 5] Both these studies, however, reported an increase in the levels of ET-1 in the BAL fluid of untreated asthmatic subjects which we did not observe in our asthmatic group (nor in the subgroup taking inhaled β_2 agonists alone), although others have observed a fall in BAL fluid levels of ET-1 at 04.00 hours in a group of patients

with nocturnal symptoms[6] which suggests that an increase of ET-1 levels in BAL fluid is not consistent in asthma. While the reproducibility and validity of sputum induction has been demonstrated,[7] and cytokines and soluble factors can be measured in induced sputum, it should be noted that samples obtained by sputum induction are not identical to those obtained by BAL.[8] The fact that ET-1 is present in sputum and saliva at levels higher than that in plasma suggests that ET-1 is produced or released locally within the respiratory tract, confirming work showing expression and secretion of ET-1 in a number of airway cells.[1]

It is not possible on the basis of the data presented to attribute any clear pathophysiological role for ET-1 in asthma, but it should be noted that patients were studied at rest, without prior bronchoconstriction. It is interesting to speculate that basal ET-1 levels may not reflect the situation in an acute exacerbation or following a bronchoconstrictor stimulus.

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