Increased leukotriene B4 and 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD

W A Biernacki, S A Kharitonov, P J Barnes

Background: Exacerbations are an important feature of chronic obstructive pulmonary disease (COPD), accounting for a large proportion of health care costs. They are associated with increased airway inflammation and oxidative stress.

Methods: Concentrations of leukotriene B4 (LTB4), a marker of inflammation, and 8-isoprostane, a marker of oxidative stress, were measured in the exhaled breath condensate of 21 patients (11 M) with COPD during an exacerbation and 2 weeks after treatment with antibiotics. In 12 patients who had no further exacerbations these markers were also measured after 2 months.

Results: LTB4 concentrations were raised during the COPD exacerbation (mean (SE) 15.8 (1.1) pg/ml and fell after treatment with antibiotics to 9.9 (0.9) pg/ml (p<0.0001). In 12 patients the level of LTB4 fell further from 10.6 (1.1) pg/ml to 8.5 (0.8) pg/ml (p<0.005) after 2 months. In 12 normal age matched subjects the LTB4 levels were 7.7 (0.5) pg/ml. Concentrations of 8-isoprostane were also increased during the exacerbation (13.0 (0.9) pg/ml) and fell after antibiotic treatment to 9.0 (0.6) pg/ml (p<0.0001). In 12 patients there was a further fall from 9.3 (0.7) pg/ml to 6.0 (0.7) pg/ml (p<0.001) after 2 months compared with normal subjects (6.2 (0.4) pg/ml).

Conclusions: Non-invasive markers of inflammation and oxidative stress are increased during an infective exacerbation of COPD and only slowly recover after treatment with antibiotics.

A study was undertaken to assess markers of inflammation (LTB4) and oxidative stress (8-isoprostane) in exhaled breath condensate in patients with exacerbations of COPD and after treatment with antibiotics. The study was conducted in a general practice clinic as most exacerbations of COPD are managed out of hospital.

METHODS

Thirty patients (19 men) with exacerbations of COPD were recruited from general practice clinics. COPD was defined as forced expiratory volume in 1 second (FEV1) of <80% predicted for age and height and a ratio of FEV1 to forced vital capacity (FVC) of <70%. In all patients the spirometric bronchodilator response to 500 µg nebulised salbutamol was <15% of the baseline when clinically stable.

All patients were current or ex-smokers; all smokers refrained from smoking for at least 12 hours before collection of breath condensate. This was confirmed by normal values of exhaled carbon monoxide levels. An exacerbation of COPD was diagnosed according to the criteria of Anthonisen and colleagues. All patients had to have the following three major symptoms: increased breathlessness, sputum volume, and purulence. We deliberately used symptoms to define exacerbations since these criteria apply in clinical practice. Patients were asked to contact practice nurses as soon as the symptoms appeared and all tests were performed within 12–16 hours.

Twelve control subjects (8 men) were recruited from the staff working in the practices. All were non-smokers and had no significant past medical history.

Study design

Patients completed a short questionnaire about their symptoms and colour of the sputum. Spirometric tests were then performed and expired breath condensate was collected. All these measurements were taken during an exacerbation,
before starting a course of a broad spectrum antibiotic (amoxycillin or erythromycin), and then repeated after 2 weeks and also after 2 months when in a stable condition. All the patients were allowed to increase the use of inhaled β₂ agonist and/or ipratropium bromide as required. However, if there was no clinical improvement after 3 days of treatment with the antibiotic they were asked to contact the doctor again.

Expired condensate was collected by an Eco Screen condenser (Jaeger, Hoechberg, Germany). Patients were required to breathe tidally through the mouthpiece connected to the condenser for 10 minutes while wearing a nose clip. Approximately 2 ml of condensate was immediately stored at –20°C, transferred on dry ice within a few days, and stored in the laboratory at –70°C.

Assays of LTB₄ and 8-isoprostane were performed by a specific immunoassay (EIA) kit (Cayman Chemical Company, Ann Arbor, Michigan, USA) as previously described. The specificity of LTB₄ and 8-isoprostane assays was 100%. The detection limit for LTB₄ was 5 pg/ml and for 8-isoprostane was 4 pg/ml. The intra-assay and inter-assay variabilities were <10%. Assays were directly validated by gas chromatography/spectrometry.

Spirometric tests were performed using a Vitalograph 2120 hand held storage spirometer (Vitalograph Ltd, Buckingham, UK). All patients were treated for 7 days with a course of a broad spectrum antibiotic (amoxycillin 500 mg tds or erythromycin 500 mg qds if they were allergic to penicillin). All patients were on inhaled corticosteroids, the dose of which remained constant during the exacerbation.

In those patients we did not repeat measurements of markers in exhaled breath condensate and we did not include the initial measurements. However, these patients had a similar degree of airways obstruction (FEV₁ 49 (18)% predicted) and similar levels of markers in initial samples of expired breath condensate (LTB₄ 16.1 (2.0) pg/ml; 8-isoprostane 14 (0.8) pg/ml).

The baseline characteristics of the 21 patients included in the analysis are summarised in table 1. Patients with COPD had a wide range of airways obstruction as shown by FEV₁. Only four patients were allergic to penicillin and they were treated with erythromycin. Since this subgroup was very small, it was impossible to make any comparison with the patients treated with amoxycillin.

All patients were on inhaled corticosteroids, the dose of which remained constant during the exacerbation, but none of those patients included in the final analysis was treated with oral corticosteroids. All the patients were allowed to increase inhaled β₂ agonist and/or ipratropium bromide as required. In all patients included in the study all three major symptoms had improved after treatment with antibiotics. We deliberately did not start oral steroids at the initial visit since, in our clinical opinion, the exacerbation was not severe enough.

All control subjects were lifelong non-smokers and they were only slightly younger than the patients with COPD (table...
treatment with antibiotics.

exhaled condensate either during the exacerbation or after
by Anthonisen

DISCUSSION

Spirometric parameters

from 9.3 (0.7) pg/ml to 6.0 (0.7) pg/ml (p<0.001, fig 2B).

exacerbations there was a further fall in 8-isoprostane levels
have repeat tests after 2 months and who had no further
(6.2 (0.4) pg/ml; p<0.005). In the 12 patients who agreed to
the level was still significantly higher than in normal subjects
(p<0.0001, fig 2A) after treatment with antibiotics, although
13.0 (0.9) pg/ml) and fell after treatment with antibiotics to
9.0 (0.6) pg/ml (p<0.005, fig 1B).

8-isoprostane

Levels of 8-isoprostane were increased during the exacerbation
periods (15.8 (1.1) pg/ml) and fell after treatment with antibiotics to
9.9 (0.9) pg/ml (p<0.0001) which was similar to the level in
normal non-smoking subjects (7.7 (0.5) pg/ml; fig 1A). In 12
patients who agreed to participate further in the study the
tests were repeated after 2 months when in a stable condition
and LTB4 levels were found to decrease further from 10.6
(1.1) pg/ml to 8.5 (0.8) pg/ml (p<0.005, fig 1B).

LTB4

LTB4 levels were increased during the exacerbation periods
and after 2 months.

8-isoprostane

levels fell after antibiotic treatment and became similar
to the levels in normal non-smoking subjects. This may reflect
a fall in neutrophil markers in the airways during the recovery
period. Furthermore, LTB4 levels fell further after 2 months in
patients who had no further exacerbations, which suggests
that exhaled LTB4 may be useful in the non-invasive
assessment and monitoring of inflammation in patients with
COPD. Our results are consistent with a recent report that
LTB4 levels are increased in the sputum of patients with bac-
terial exacerbations of COPD and fall rapidly after antibiotic
treatment. In another study, which included larger numbers
of patients with exacerbations of COPD, the LTB4 level was
increased but fell after treatment and remained stable for 56
days. The increased concentration of LTB4 in patients with
COPD is correlated with myeloperoxidase activity, indicating
activation of neutrophils. This relationship may reflect
increased numbers of bronchial neutrophils in these patients.
A similar increase in LTB4 is also seen in patients with bronchiectasis. Infection may also increase the production of
other inflammatory mediators—for example, IL-8, IL-6, and
TNF-α levels in induced sputum are increased during exacer-
ations of COPD—and these measurements can be used to
evaluate the severity of airway inflammation. Endothelin-1
(ET-1) has also been shown to increase in the sputum during
COPD exacerbations.

Infection is the most common cause of exacerbations of
COPD and may be responsible for an increase in inflammation
in the airways. Indeed, in a recent study an increased airborne
bacterial load was strongly related to several markers of
inflammation in the sputum including IL-8 and LTB4. Sputum
levels of IL-8 and TNF-α were reported to increase
significantly during exacerbations of COPD and to return to
baseline after recovery.

Our study also showed that exhaled 8-isoprostane levels
were increased during exacerbations. This may reflect
increased oxidative stress. Indeed, an infection may contribute
to oxidative stress, which is a major component of airways

1). None had a significant medical history, they were not tak-
ing any medication, and they had normal spirometric values.
All control subjects were living in the same area as the study
patients.

LTB4

LTB4 levels were increased during the exacerbation periods
(15.8 (1.1) pg/ml) and fell after treatment with antibiotics to
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Spirometric parameters

There was no significant change in FEV1, after treatment with
antibiotics (initial measurement 1.15 (0.08) l, after treatment
1.25 (0.08) l, p>0.05). Furthermore, in 12 patients who were
followed up for 2 months there were no significant changes in
FEV1, (initial FEV1, 1.23 (0.11) l, after 2 weeks 1.14 (0.14) l
after 2 months 1.26 (0.18) l). There was no significant
correlation between the degree of airways obstruction as
shown by FEV1 and the levels of LTB4 or 8-isoprostane in the
exhaled condensate which return to
normal during the recovery period. LTB4 is a potent and selec-
tive chemoattractant of neutrophils and may be released
from macrophages and epithelial cells as well as from
activated neutrophils. The increase in LTB4 may contribute
to the increase in neutrophil influx during an exacerbation.
LTB4 levels fell after antibiotic treatment and became similar
to the levels in normal non-smoking subjects. This may reflect
a fall in neutrophil markers in the airways during the recovery
period. Furthermore, LTB4 levels fell further after 2 months in
patients who had no further exacerbations, which suggests
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Figure 2 (A) Concentrations of exhaled 8-isoprostane during an exacerbation of COPD (n=21) and after 2 weeks compared with normal
non-smoking subjects (n=12). (B) Concentrations of exhaled 8-isoprostane in 12 patients with COPD during an exacerbation, after 2 weeks,
and after 2 months.
Inflammation and oxidative stress in infectious exacerbations of COPD

This study confirms the important role of LTB4 in inflammation in patients with exacerbations of COPD. LTB4 in exhaled condensate is increased during a COPD exacerbation and may play a part in mediating airway inflammatory changes. Oxidative stress, as assessed by the level of 8-isoprostane in exhaled condensate, is also increased during exacerbations of COPD, but appears to take longer to return to normal after antibiotic treatment. Measurements of LTB4 and 8-isoprostane in exhaled condensate may provide another useful diagnostic tool for detecting and monitoring inflammation and oxidative stress in patients with COPD. They can be performed repeatedly even in patients with severe airways obstruction and may be helpful in detecting exacerbations of COPD.

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The cost of helical CT lung cancer screening is high


In this study a computer simulated model of a hypothetical cohort of 100 000 60 year old current, quitting, and former smokers was used to assess the cost effectiveness of helical CT lung cancer screening. Using the best available data on outcomes associated with lung cancer screening, the costs of diagnosis, treatment and long term outcomes, the efficacy of changing the clinical stage distribution of lung cancer so that the screened group would have fewer advanced staged cancers and more localised stage cancers was assessed. Biases such as lead time (from earlier onset of costs as a result of earlier diagnosis), length (due to long latency periods), and overdiagnosis (for example, of subclinical disease) were incorporated. Over 20 years there were 553 fewer lung cancer deaths and 1186 false positive invasive tests in the screened group. The incremental cost for current, quitting, and former smokers was $116 300, $558 600, and $2 322 700 per quality-adjusted life year (QALY) gained, respectively. Even the most favourable parameters estimated the most cost effective outcome for current smokers to be $42 500 per QALY.

The debate on the usefulness of helical CT scanning to screen for lung cancer rages on. The National Cancer Institute has recently initiated a large randomised trial to assess the efficacy of helical CT screening for detection of early lung cancer. While the results of this trial are awaited, commercially available cancer screening programmes are being widely promoted in the US. Mahadevia and colleagues have used complex analysis techniques to suggest that screening is not cost effective with current methods.

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