THE ROLE OF A SOLUBLE TNF-α RECEPTOR FUSION PROTEIN (ETANERCEPT) IN CORTICOSTEROID-REFRACTORY ASTHMA: A Double Blind, Randomised Placebo-Controlled Trial

JB Morjaria, MRCP, MD (1)
AJ Chauhan, FRCP, PhD (2)
KS Babu, MRCP, MD (1)
R Polosa, MD, PhD (3)
DE Davies, PhD (1)
ST Holgate, F Med Sci, DSc (1)

AFFILIATIONS

(1) Infection, Inflammation and Repair Division, Southampton University Hospitals Trust, Level F, Mailpoint 810, South Academic Block, Southampton, SO16 6YD, UK.

(2) Dept of Respiratory Medicine, Queen Alexandra Hospital, Portsmouth, PO6 3LY, UK.

(3) Dipartimento di Medicina Interna, Universita' di Catania, Presidio Ospedaliero Ascoli-Tomaselli, 95125 Catania, ITALY
CORRESPONDING AUTHOR

<table>
<thead>
<tr>
<th>Dr J B Morjaria</th>
<th><a href="mailto:jbm@soton.ac.uk">jbm@soton.ac.uk</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>IIR, Maipoint 810, South Academic Block</td>
<td></td>
</tr>
<tr>
<td>Southampton General Hospital</td>
<td></td>
</tr>
<tr>
<td>Tremona Road</td>
<td>+44 (0)1279 653227</td>
</tr>
<tr>
<td>Southampton SO16 6YD</td>
<td>+44 (0)2380 701771</td>
</tr>
</tbody>
</table>

License for Publication

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive license (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article to be published in THORAX editions and any other BMJPGL products to exploit all subsidiary rights, as set out in our license (http://thorax.bmj.com/ffora/licence.pdf).

Sources of Support

This study was supported by an educational grant from Wyeth Pharmaceuticals, UK, and they were not sponsors of the study. The trial was investigator-initiated and the sponsors were not involved in the study design, data collection, analysis or interpretation of the data. A copy of the manuscript was nonetheless sent to Wyeth before submission.

Running Head

Severe asthma, etanercept, TNFα.

Word count

Abbreviations

PEF=Peak Expiratory Flow
FEV₁=Forced Expiratory Volume in 1s
FVC=Forced Vital Capacity
OCS=Oral Corticosteroid
ICS=Inhaled Corticosteroid
TNFα=Tumour Necrosis Factor α
CRP=C-Reactive Protein
AQLQ=Asthma Quality of Life Questionnaire
ACQ=Asthma Control Questionnaire
BALF=Bronchoalveolar lavage fluid
URTI=Upper respiratory Tract Infection
RCT=Randomised Controlled Trial

Normal Range

The normal range of serum C-reactive protein and albumin described in this study are <7.5 mg/L and 35-48 gm/L in healthy adults.

Importance of Study

This is the first parallel group RCT of once weekly etanercept in severe asthma, showing small but significant improvements in asthma control and systemic inflammation in favour of etanercept, but not in lung function or bronchial hyperresponsiveness compared to placebo. These potential benefits need to be tested in larger multi-centre controlled trials.
ABSTRACT

Rationale

TNF-α is a cytokine recognized as a therapeutic target in chronic inflammatory diseases.

Objectives and Methods

We report a randomised double-blind placebo-controlled parallel group trial of etanercept (an IgG1-TNF p75 receptor fusion protein), administered once weekly for 12 weeks in 39 patients with severe corticosteroid refractory asthma. Efficacy was measured by change from the pre-treatment baseline in Asthma Related Quality of Life (AQLQ) and Asthma Control (ACQ) Questionnaires scores (the primary end-points), lung function, PEF and bronchial hyperresponsiveness (BHR). We also assessed sputum and serum inflammatory cells and cytokines, serum albumin and C-reactive protein as biomarkers of inflammation.

Main results

There was a small but significant difference in reduction of ACQ scores between treatment and placebo (-1.11 (95%CI -1.56, -0.75) and -0.52 (95%CI -0.97, -0.07) respectively, p=0.037). There was no significant difference in improvements in AQLQ scores, lung function, PEF, BHR and exacerbation rates between groups. Minor adverse events including injection site pain and skin rashes were more frequent with etanercept. There was a significant reduction in sputum macrophages and CRP, and increases in serum TNF-α and albumin following treatment, but not in other laboratory parameters.
Conclusion

Etanercept therapy over 12 weeks demonstrated only a small but significant improvement in asthma control and systemic inflammation as measured by serum albumin and CRP. Larger randomised placebo-controlled trials are required to clarify the role of TNF-α antagonism in subjects with severe refractory asthma.
INTRODUCTION

Asthma is a disorder of the conducting airways characterised by variable airflow obstruction and increased bronchial hyperresponsiveness (BHR) to a range of environmental stimuli. In the majority of patients, mild to moderate asthma airway dysfunction is usually responsive to inhaled corticosteroids that form the mainstay of therapy. As the disease becomes more severe and chronic, it adopts a more aggressive phenotype with evidence of neutrophil infiltration and airway wall remodelling. Asthmatic patients with this severe phenotype respond poorly to standard treatments and many are not adequately controlled despite regular systemic corticosteroids and good adherence. We have proposed that these changes in asthma phenotype may reflect expression of an additional Th1 inflammatory profile with increased production of TNF-α, a multifunctional cytokine that augments the activity of many cells implicated in asthma pathogenesis including immune, inflammatory, smooth muscle and epithelial cells. A case has been made for TNF as a mediator of asthma in humans where inhaled TNF-α causes transient BHR and an influx of neutrophils into the airways. Genetic polymorphism of the TNF-α gene on chromosome 6 is also associated with asthma, its accompanying severity and BHR.

The potential importance of TNF in severe refractory asthma has recently been strengthened by two small clinical trials of etanercept, a p75 IgG1 fusion protein that binds both TNF-α and TNF-β thereby preventing interaction with its cell-bound high (p75) and low (p55) affinity receptors. In the first open labelled study on 15 patients, etanercept (Enbrel®) 25mg administered subcutaneously twice weekly for 12 weeks led to a marked reduction in symptoms and BHR but only moderate changes in baseline FEV1. The second, a crossover placebo controlled study on 10 refractory asthmatics using the same regimen for 10 weeks produced almost identical findings, but in addition revealed a substantial increase in asthma-
related quality of life (AQLQ) and that circulating mononuclear cell membrane-associated TNFα was not only selectively elevated in severe asthma but also predicted the clinical response to etanercept. A third parallel group RCT of the TNF-blocking antibody Infliximab in less severe asthma revealed significant protection against exacerbations of asthma and other endpoints, but failed to achieve significance in the primary endpoint of morning PEF.

In other chronic inflammatory diseases such as rheumatoid arthritis and psoriasis, TNF blockade has been especially effective in relieving systemic manifestations of the disease measured by changes in quality of life, fatigue, anxiety and depression scores. Here we describe a parallel group RCT with etanercept in severe refractory asthma in which asthma control and related quality of life was selected as the primary clinical end points. We also measured a number of biomarkers of inflammation in both blood and sputum.

MATERIALS AND METHODS

Patients

The majority of patients were recruited from 2 difficult asthma clinics at Southampton General and Queen Alexandra Hospitals, and others from Dorset General and St. Mary’s Isle of Wight Hospitals, UK. Asthma was confirmed by the presence of objective assessments variable airflow obstruction and/or bronchial hyperresponsiveness. This included an increase in FEV₁ by at least 12% after the inhalation of 400 µg of salbutamol delivered by a metered dose inhaler and spacer the concentration of methacholine required to cause a 20% (PC20) reduction in the forced expiratory volume in one second (FEV₁) of <8 mg/ml, the latter only if subjects had a predicted FEV₁ of >50%. All patients also met the ‘Global Initiative for Asthma’ criteria for severe refractory asthma, which included current treatment with oral
prednisolone (2-30 mg/day) and/or high dose inhaled corticosteroids (>2000 mcg/day beclomethasone equivalent) and long acting β₂-adrenoceptor agonists. A high proportion of patients received one or more of theophyllines, leukotriene receptor antagonists and nebulised salbutamol. Current smokers, subjects with a smoking history of >10 pack years, other co-existing lung disease, a history of tuberculosis, multiple sclerosis, systemic lupus erythematosus or other autoimmune diseases were excluded. Patients were also excluded from participation if they had a history of URTI within 2 months, evidence of tuberculosis (PPD skin test >10 mm, or positive chest x-ray), history of opportunistic infections within the previous 6 months, previous malignancy and/or history of lymphoproliferative disease. All patients were considered compliant with treatment by cross-checking GP prescription records or measurement of serum theophylline levels as appropriate on a central primary and secondary care database. Patients thought to have uncontrolled asthma as a consequence of co-existent conditions such as gastro-oesophageal reflux, rhinitis or occupational triggers were also excluded. The subjects were asked not to alter any regular controller asthma medications during the study, though they were allowed to adjust reliever medication use as necessary. The patient characteristics at baseline are shown in Table 1.
Table 1
The characteristics of patients at baseline

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>PLACEBO N=20</th>
<th>ETANERCEPT N=19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>N=20</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>N=19</td>
<td>44.0</td>
<td>39.0</td>
</tr>
<tr>
<td>Duration of Asthma (yrs)</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>N=18</td>
<td>30.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Corticosteroid use</td>
<td>OCS and ICS</td>
<td>ICS alone</td>
</tr>
<tr>
<td>N=13</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Nebulised β2 agonist use</td>
<td>Number</td>
<td>Mean daily dose (mg/day)</td>
</tr>
<tr>
<td>N=8</td>
<td>7</td>
<td>13.6</td>
</tr>
<tr>
<td>Oral theophylline use</td>
<td>Number</td>
<td>Mean daily dose (mg/day)</td>
</tr>
<tr>
<td>N=9</td>
<td>10</td>
<td>612.5</td>
</tr>
<tr>
<td>Antidepressant use</td>
<td>Number</td>
<td></td>
</tr>
<tr>
<td>N=11</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Asthma Control</td>
<td>AQLQ (Total Score)</td>
<td>3.56±1.15</td>
</tr>
<tr>
<td></td>
<td>ACQ (Total Score)</td>
<td>3.07±0.74</td>
</tr>
<tr>
<td>Lung Function</td>
<td>Actual FEV1 (Litres)</td>
<td>1.84±0.78</td>
</tr>
<tr>
<td></td>
<td>% predicted FEV1</td>
<td>58.8±17.8</td>
</tr>
<tr>
<td></td>
<td>Forced Expiratory Ratio (FEV1/FVC)</td>
<td>0.63±0.12</td>
</tr>
<tr>
<td></td>
<td>FEV25-75 (%)</td>
<td>1.16±0.78</td>
</tr>
<tr>
<td>Peak Expiratory Flow (PEF)</td>
<td>Morning PEF (L/min)</td>
<td>302±88</td>
</tr>
<tr>
<td></td>
<td>Evening PEF (L/min)</td>
<td>314±89</td>
</tr>
<tr>
<td></td>
<td>Diurnal Variation in PEF</td>
<td>30.2±28.5</td>
</tr>
<tr>
<td></td>
<td>Average Daily PEF (L/min)</td>
<td>308±87</td>
</tr>
<tr>
<td>PC20 ‡ (mg methacholine/ml)</td>
<td>2.49±4.05</td>
<td>1.92±2.07</td>
</tr>
<tr>
<td>Serum Measurements</td>
<td>Eosinophils (x10⁶/L)</td>
<td>0.53±0.38</td>
</tr>
<tr>
<td></td>
<td>Neutrophils (x10⁶/L)</td>
<td>5.24±2.05</td>
</tr>
<tr>
<td></td>
<td>Albumin (gm/L)</td>
<td>39.9±3.57</td>
</tr>
<tr>
<td></td>
<td>CRP (mg/L)</td>
<td>5.24±4.12</td>
</tr>
<tr>
<td></td>
<td>TNFα (pg/ml)</td>
<td>2.49±1.31</td>
</tr>
<tr>
<td></td>
<td>Total IgE (IU/ml)</td>
<td>395±566</td>
</tr>
<tr>
<td>Sputum Cells</td>
<td>* Eosinophils (%)</td>
<td>0.10 (0.06, 0.21)</td>
</tr>
<tr>
<td>(n=8 both groups)</td>
<td>* Neutrophils (%)</td>
<td>0.40 (0.30, 0.53)</td>
</tr>
<tr>
<td></td>
<td>* Macrophages (%)</td>
<td>0.44 (0.23, 0.55)</td>
</tr>
<tr>
<td>Sputum Cytokines</td>
<td>* IL-6 (pg/ml)</td>
<td>43.1 (24.35, 80.1)</td>
</tr>
<tr>
<td>(n=8 both groups)</td>
<td>* IL-8 (pg/ml)</td>
<td>1696.2 (1157.9, 2282.5)</td>
</tr>
<tr>
<td></td>
<td>* IL-1β (pg/ml)</td>
<td>0 (0, 54.4)</td>
</tr>
</tbody>
</table>

All values are means±SD unless stated. ‡ n=13 in placebo and n=11 in etanercept group. *Values are median (IQR).
Study Design

This was a double blind parallel-randomised placebo-controlled trial, which was approved by the SE Hampshire and Isle of Wight Research Ethics Committee, and all patients provided informed consent. The trial was investigator-initiated and the sponsors were not involved in the study design, data collection, analysis or interpretation of the data. Sample size methods are given in the Appendix. Patients attended the clinic for a screening visit followed by an entry visit after a minimum 2-week run in period for baseline measurements and to confirm eligibility. The study plan is given in Figure 1. Patients were randomly allocated at Visit ‘0’ to receive 50 mg of etanercept or matched placebo by subcutaneous injections once a week for 12 weeks as add on therapy to their current medication. Selection of the dose and duration of treatment were chosen from initial proof of concept studies of etanercept in severe asthma and from the recent observation in patients with active rheumatoid arthritis. Weekly physiological measurements and diary card collections were made on the day of the visit for treatment, which was administered by the investigators/nursing staff. Randomisation was undertaken by Wyeth Pharmaceuticals (Taplow, Berks, UK) using a permuted blocks of four method. All patients were followed up 4 weeks after completion, and no change was made to the subjects’ regular asthma medication during the 12 weeks treatment period.

Asthma Quality of Life and Control Questionnaires

The AQLQ was assessed at baseline and at the end of the treatment phase, and the ACQ at each weekly visit. A lower AQLQ score indicated increased impairment, and a higher ACQ score indicated worsening asthma control (questionnaire details are covered in the Appendix).
Lung Function

Baseline lung function was recorded as forced expiratory volume in one second (FEV₁), forced vital capacity (FVC) and expiratory ratio (FEV₁/FVC) using a Vitalograph® Compact Spirometer (Vitalograph Medical Instrumentation, Buckingham, UK) at baseline and at weekly intervals throughout the trial. The forced expiratory flow between 25% and 75% of FVC (FEV₂₅-₇₅, using Vitalograph® Electronic Compact II spirometer, Vitalograph Medical Instrumentation, Kansas, USA) was measured at baseline and end of treatment. Daily diary cards were used to monitor morning and evening peak expiratory flow (PEF) using the Wright mini peak flow meter (Clement Clark International Limited, Harlow, UK).

Bronchial Hyperresponsiveness (BHR)

BHR to inhaled methacholine was measured using the 5 breath procedure (described by Chai et al. ¹²) at visits 0 and 12. For safety reasons, the test was conducted only on subjects whose FEV₁ was >50% predicted. Detailed methods are given in the Appendix. The effect of treatment on BHR was expressed as the change in doubling dilutions of methacholine required to achieve a 20% fall in FEV₁ before and after 12 weeks treatment, logarithmically transformed and a group mean derived.

Sputum and Serum Biomarkers of Inflammation

Venous blood collected at baseline and at the end of the treatment was stored at -80°C. Sputum induction was performed as previously described with aerosolized hypertonic (4.5%) saline using an ultrasonic nebuliser (Devilbliss Ultraneb 2000, PA, USA). ¹³ For safety
reasons, sputum induction was not undertaken on subjects with a baseline FEV$_1$ <45% of predicted. Details methods of sputum and serum markers are enclosed in the Appendix.

**STATISTICAL ANALYSES**

The primary end points were the differences in change in the mean score from baseline (visit 0) and end of treatment (visit 12) on the AQLQ, and change in mean scores from baseline and the last 2 treatment visits (visits 11 and 12) for the ACQ. Secondary end-points were similar differences from baseline to visit 12 for BHR, and to the last 2 treatment visits for predicted FEV$_1$, FEV$_1$/FVC, morning, evening and average daily PEF, and diurnal variation in PEF (calculated by the difference in the evening and morning PEF values). Differences from baseline for laboratory parameters in sputum and serum were similarly analysed.

The distributions were assessed for normality by Shapiro-Wilks tests and measurement of equality of variance ratios by F-tests. Differences between groups in clinical and laboratory outcomes with a normal distribution were compared using unpaired Students T-tests or Mann-Whitney U-tests for non-normal distributions. The data were analysed using Stata version 8 (Stata Corporation, College Station, Texas, USA) and Statview Version 5.1 (SAS Institute Inc., Cary, NC). For missing data the intention to treat (ITT) methodology was used with missing values being ascribed by Last Observation Carried Forward (LOCF).

The proportion of patients experiencing exacerbations, adverse events or withdrawing from nebulised β$_2$-agonists between treatment and placebo groups were compared by 2x2 contingency tables using Chi-squared tests. We also explored relationships between baseline characteristics, sputum or serum markers, or changes in these parameters with the changes in the primary and secondary end-points by analyses of variance.
RESULTS

Baseline Characteristics

A total of 59 patients were screened, 39 were enrolled into the study, and 19 patients were randomised to active treatment. Overall, there were 20 patients who were excluded after screening (Figure 2). Details of age, gender, duration of asthma, lung function at baseline medication usage and other characteristics are shown in Table 1. There were three withdrawals, two in the placebo group at weeks 4 and 8 due to ‘ineffectiveness of treatment’ and one in the etanercept group at week 10 due to work-related stress. Overall, the comparison of baseline values of AQLQ, ACQ, lung functions and PC20 were not significantly different between the two treatment groups (p>0.05 for all variables, Table 1). Data for AQLQ, ACQ and lung function before and at the end of treatment was available in all patients completing the study. Missing diary card data for PEF was <5%.

Asthma Quality of Life and Control Questionnaires

Of the two primary end-points, there were increases in mean AQLQ scores for both treatment (1.03, 95% CI 0.64, 1.39) and placebo (0.68, 95% CI 0.14, 1.23) but the difference between groups was not significant (p=0.084), shown in Figure 3(a) and Table 2. There was however a significant difference in reductions in mean ACQ scores between treatment (-1.11 (95% CI -1.56, -0.75) and placebo (-0.52 (95% CI -0.97, -0.07), p=0.030 (Figure 4(a) and Table 2).
Table 2

The changes in clinical and laboratory outcomes from baseline after 12 weeks of active treatment or placebo. N is the number of patients or paired serum and sputum samples available for analysis for clinical or laboratory outcomes respectively.

<table>
<thead>
<tr>
<th>CLINICAL OUTCOMES</th>
<th>PLACEBO</th>
<th>ETANERCEPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Before</td>
</tr>
<tr>
<td>AQLQ Score (0-7)</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>ACQ Score (0-7)</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Lung Function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual FEV₁ (Litres)</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>% predicted FEV₁</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Expiratory Ratio (FEV₁/FVC)</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>FEV₂₅₋₇₅</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Peak Expiratory Flow (PEF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning PEF (L/min)</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Evening PEF (L/min)</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Diurnal Variation in PEF</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Average PEF (L/min)</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>PC20 ‡(mg methacholine/ml)</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>†Doubling dilutions (mg/ml)</td>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>
### (continued)

<table>
<thead>
<tr>
<th></th>
<th>PLACENPO</th>
<th>ETANERCEPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td><strong>Cells, Inflammatory Markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Eosinophils (x10⁶/L)</strong></td>
<td>19</td>
<td>0.530</td>
</tr>
<tr>
<td><strong>Neutrophils (x10⁶/L)</strong></td>
<td>19</td>
<td>5.270</td>
</tr>
<tr>
<td><strong>Albumin (gm/L)</strong></td>
<td>17</td>
<td>38.36</td>
</tr>
<tr>
<td><strong>CRP (mg/L)</strong></td>
<td>15</td>
<td>5.245</td>
</tr>
<tr>
<td><strong>TNFα (pg/ml)</strong></td>
<td>18</td>
<td>2.490</td>
</tr>
<tr>
<td><strong>Total IgE (IU/ml)</strong></td>
<td>17</td>
<td>357.1</td>
</tr>
<tr>
<td><strong>SPUTUM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td><strong>Cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Eosinophils (%)</strong></td>
<td>8</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Neutrophils (%)</strong></td>
<td>8</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Macrophages (%)</strong></td>
<td>8</td>
<td>0.44</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IL-6 (pg/ml)</strong></td>
<td>8</td>
<td>43.10</td>
</tr>
<tr>
<td><strong>IL-8 (pg/ml)</strong></td>
<td>8</td>
<td>1696.2</td>
</tr>
<tr>
<td><strong>IL-1β (pg/ml)</strong></td>
<td>8</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The table refers to the differences in change in outcome between the mean of visit 0 and mean of visits 11 and 12 for all outcomes except AQLQ, PC20, FEV_{25-75}, and sputum and serum samples, which refer to the change between visit 0 and 12.

† The doubling dilution is the difference in the log PC20 before and after treatment divided by log 2.
‡ Geometric Mean calculation
* Non-parametric Mann-Whitney U-tests used, all others are Students t-tests.
Lung Function and PEF

There were small increments in FEV₁, %predicted FEV₁ and FEV₂₅₋₇₅ in the etanercept group compared to placebo (where there were corresponding decrements), but the differences between groups were not significant (Table 2). There were no significant differences in change in morning or evening PEF, or diurnal variation in PEF. The forced expiratory ratio (FEV₁/FVC) improved in both groups over the study period though the difference was not significant. The average daily PEF (L/min) also improved over the study period though the difference in improvements between groups was not significant (Figure 4(b) and Table 2).

Bronchial Hyperresponsiveness

Methacholine bronchoprovocation was performed in 13 patients in the placebo and 11 patients in the etanercept group. There was a greater improvement in PC20 in favour of etanercept compared to placebo, also when expressed as doubling dilutions of methacholine, but the differences between groups were not significant (Figure 3(b) and Table 2).

Sputum and Serum Biomarkers of Inflammation

Sputum analyses were performed in 8 patients in each group (Table 2). The differences in numbers of paired serum and sputum samples available for analysis relate to difficulties in venous sampling, quality of specimens, and prioritisation of tests for less sufficient clinical samples. Serum TNF-α levels were very low at baseline in most of the patients studied; but increased dramatically after 12 weeks of etanercept but not placebo treatment (p<0.001, Table 2). There was a significant reduction in CRP in the etanercept group (-1.162 mg/L (95%CI -2.003, -0.321)) compared to placebo (-0.286 gm/L (95%CI -1.706, 1.134), p=0.029 (Figure 3(c)), and a significantly greater improvement in serum albumin in the etanercept
group (1.00 gm/L (95%CI 0.12, 1.87)) compared to placebo (-0.47 gm/L (95%CI -1.43, 0.49), p=0.022, (Figure 3(d)), also shown in Table 2.

Levels of IL-6 and IL-1β in sputum supernatants were reduced following treatment in the etanercept group but the differences between groups were not significant. There was a small but non-significant increase in sputum eosinophils and neutrophils in the etanercept group. There was a significant reduction in sputum macrophages in the etanercept group (-0.09 x 10^6/gm (IQR -0.265, -0.008) compared to placebo (0.11 x 10^6/gm (IQR -0.033, 0.195)), p=0.017 (Table 2).

Other Events

There were 7 subjects in each group who required regular high dose nebulised salbutamol (Table 1). Four subjects in the etanercept group voluntarily discontinued their nebulised bronchodilators by the end of the study, compared to none in the placebo group (p=0.018). There were equal numbers of asthma exacerbations and upper respiratory tract infections in both treatment groups. Other reported adverse events were mild, but local injection site reactions and skin rashes were more common in the etanercept group (p=0.014 and p=0.030 respectively). One patient in each group was admitted to hospital for an exacerbation of asthma, with a hospital length of stay of less than 48 hrs for both. There were no other serious adverse events. Details on adverse events are in the Appendix.
Follow Up

The majority of patients had changes in the AQLQ and ACQ scores, and reductions in daily PEF, and FEV₁ commensurate with deterioration in asthma control and quality of life 4 weeks after the study, but the differences between groups were not significant (data not shown). There were also no serious or minor adverse events at follow up.

DISCUSSION

This study of etanercept in patients with severe refractory asthma has shown an improvement in one of our two primary end-points. There was a small but significant improvement in asthma control assessed by questionnaire (ACQ) but not asthma-related quality of life (AQLQ) after 12 weeks of treatment. There were no improvements in the secondary end-points of lung function, PEF or bronchial hyperresponsiveness (in the small number of subjects in whom challenge was possible). There were no serious adverse events, though minor injection site reactions and skin rashes were more common in the etanercept group, and the rate of exacerbations of asthma was the same in both groups.

It is possible that those patients in the etanercept group reporting voluntary withdrawal of nebulised bronchodilators at the end of the study (compared to none on placebo) could have augmented the observed improvement in AQLQ and ACQ scores. The time course over which changes after etanercept therapy may occur are not known, though based on our previous study and a recent small cross-over trial of etanercept in severe refractory asthma which demonstrated improvements after 10 weeks of therapy, we sought to examine similar changes in outcome after 10 weeks by comparing differences from baseline with the mean of weeks 11 and 12, rather than week 12 alone. The treatment duration was chosen based on the previous trials with etanercept ⁴ ⁸ and initial trials in rheumatoid arthritis where etanercept
was used as a treatment option for 12 weeks. Also, the administration of treatment once weekly was based from prior studies in RA. The majority of patients were recruited mainly from 2 centres, and assessment of recruitment site showed no interaction with any of the outcomes or treatment effects in analyses of covariance with a full effects model (data not shown).

Improvements in the primary and secondary end-points were seen in both placebo and treatment groups, though only differences in ACQ scores were significantly different between groups. A within-group change of 0.5 in scores on the ACQ (and AQLQ) is considered clinically significant and we found a between-group difference of 0.59. Increased compliance with treatment upon recruitment into the study is unlikely to explain the discrepancy as we checked compliance by examining prescription records, clinic attendances and pre-enrolment blood tests, and excluded 2 patients in the screening period in whom non-adherence to therapy was thought to be significant. Alternatively, it is probable that patients affected by depression as a result of their underlying chronic medical condition are more likely to experience high placebo response rates (see post-hoc analyses in appendix). When the observations were restricted to patients not taking antidepressants, the differences in improvement in both ACQ and AQLQ scores became more significant between etanercept and placebo compared to differences when the groups were combined. We observed between group differences in ACQ and AQLQ scores of 0.93 and 0.58 respectively, both above the minimal important changes. Despite the potential limitations of such a post-hoc analysis, this finding leads us to suggest that future trials of severe refractory asthma should evaluate co-existent depression and anxiety in this sub-group of asthma patients, in addition to asthma-specific measures such as the AQLQ and ACQ.
We had calculated the sample size in our study on expected differences in AQLQ and ACQ based on our previous study, but a large proportion of patients enrolled into the study remained too severe to have safely performed methacholine challenge. Therefore, the effect on methacholine BHR may have been underestimated due to the small numbers, even though some patients in the etanercept group showed significant ‘within patient’ differences. It is possible that the improvements in lung function were also not demonstrated because the sample size was calculated on other primary end points. Furthermore, it is unlikely we included any patients with hitherto unrecognised COPD as the diagnosis of asthma was confirmed in each patient and smokers were excluded. However, we studied asthmatic patients with very severe airflow obstruction (mean % predicted FEV1 58-59%) and it is possible that we included some with ‘fixed’ airflow obstruction as we did not perform assessments of ‘corticosteroid responsiveness’ in the screening or recruitment period. If the airway obstruction was relatively fixed this would reduce the possibility of any improvement. Another possible explanation is that the study measured the post-bronchodilator FEV1 at each visit, and if patients confirmed having taken a short acting β2-adrenoceptor agonist within an hour of the visit, a further bronchodilator was not administered again; we therefore could not prove with certainty that every measurement of FEV1 was after a bronchodilator.

The disparity between impacts on quality of life and disease control in severe asthma and lack of efficacy in lung function has been highlighted in clinical trials of other biological therapies such as anti-IgE treatment. We have similarly demonstrated significant improvements in asthma control and quality of life (in patients’ not on antidepressants), but not lung function. Trials of etanercept in other chronic diseases such as rheumatoid arthritis and psoriasis have also revealed a disproportionate effect on symptoms or quality of life scores raising the possibility that in these disorders as well as in asthma, TNF-α generated in...
the inflamed organ may have systemic or extra-pulmonary effects\textsuperscript{21} and that the discrepancy between quality of life and lung function in severe asthma have different mechanisms of effect.\textsuperscript{22} We measured the acute-phase reactants CRP and albumin as surrogates of systemic inflammatory activity, and confirmed that levels of CRP decreased by a mean 1.162 mg/L and serum albumin increased by a mean 1gm/l in the etanercept group compared to placebo. Both changes occurred within the normal range for healthy adults, using robust assays applicable to most hospital laboratories, even though it is recognised that standard assays for CRP may not be sensitive enough to detect levels of systemic inflammation close to the normal range, as in this study. Other studies have recently reported elevated levels of CRP in mild ‘corticosteroid naive’ asthma\textsuperscript{23} and COPD,\textsuperscript{24} and further investigation of this effect in severe asthma using high sensitivity CRP assays are warranted. Our results indicate reduced systemic inflammation with etanercept therapy in asthma, in keeping with similar observations of reduced CRP in rheumatoid arthritis and ankylosing spondylitis after TNF blockade.\textsuperscript{25,26} In this study, the baseline serum CRP or albumin did not predict any of the clinical responses. Overall, these observations support the view that refractory asthma may be a systemic inflammatory disorder and that TNF blockade may be important in the systemic manifestations of the disease.

Despite previously having shown that TNF-\(\alpha\) is increased in bronchoalveolar lavage fluid (BALF) and bronchial biopsies in patients with severe asthma,\textsuperscript{4} in keeping with others,\textsuperscript{27} we were unable to detect any TNF-\(\alpha\) in the sputum of patients despite the high sensitivity and specificity of the assays. One explanation for this is the effects of dithioerythritol or other media used in recovery of mediators in sputum, which can reduce cytokine recovery.\textsuperscript{28} We were able to detect other cytokines (IL-6, IL-8 and IL-1\(\beta\)) in preference to TNF suggesting this effect of laboratory processing less likely. We did nevertheless detect highly immuno-
reactive TNF-α in the serum after etanercept therapy compared to placebo which most likely represents TNF-α bound to the etanercept fusion protein, but not in the sputum following treatment. This demonstrates that while we were unable to detect this cytokine in the circulation at baseline, etanercept therapy was very effective in binding to TNFα to render it biologically inactive and is consistent with similar observations in BALF of patients with mild asthma treated with etanercept. Previous studies have suggested that elevated levels of IL-6 and IL-1β in BALF is indicative of symptomatic asthma, and in this study levels of IL-6, IL-8 and IL-1β were all reduced in sputum of patients treated with etanercept compared to placebo, though none were significant. This is consistent with our previous observations and that of others. One point of interest is that sputum macrophages were significantly reduced in the etanercept group compared to placebo, while small but non significant increases in eosinophils and neutrophils were observed in patients after etanercept therapy. The level of sputum macrophages at baseline did not consistently influence any of the main outcomes in the small numbers of patients with available data.

Etanercept treatment was generally well tolerated with no serious adverse events other than one patient in each group admitted to hospital for an exacerbation of asthma. There was however a small increase in local injection site and skin reactions in the etanercept group. We also evaluated the number of asthma exacerbations and upper respiratory infections, and did not find any differences between groups, in contrast to another recent study of anti-TNF therapy in less severe asthma.

In summary this randomised controlled trial adds some support for the role of TNF-α in the pathogenesis of severe corticosteroid refractory asthma. It confirms small improvements in asthma control and systemic inflammation after 12 weeks of etanercept therapy compared to
placebo. Larger multi-centre, placebo-controlled RCTs adequately powered for each clinical outcome especially BHR, are now required to evaluate this therapeutic option in patients with severe asthma.

ACKNOWLEDGEMENTS

We thank Lesley-Ann Vickers, Louisa Little, Malcolm North, Sandy Smith and Sumita Kerley, for their invaluable assistance in conducting this trial. We are also grateful to Dr D. Murphy and his team at the St Mary’s Hospital, Isle of Wight. We are thankful to Wyeth Laboratories for providing the drugs (placebo and etanercept) free of cost and for the educational grant for the clinical research fellow (JM) and nursing staff. We are grateful to Drs LC Lau and J Ward for help with the laboratory analyses, and to Drs P Vijayanand and D Bagmane for their help in clinical supervision during the study. We would also like to thank Mr B. Higgins from The Department of Mathematics, University of Portsmouth for guidance on the statistical analyses. We also thank Dr H Arshad and Professor R. Djukanovic for help in planning this study. STH is a UK Medical Research Council funded Clinical Professor.

CONFLICTS OF INTEREST

This study was performed with an educational grant from Wyeth Pharmaceuticals. All authors confirm (except JM and STH, see below) they are not involved in any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this manuscript. JM was funded by the educational grant to conduct this study. AJC in the last 5 years has received research funding, honoraria for lectures and educational grants from Astra Zeneca, Glaxo Smith Kline, Boehringer Ingelheim and Merck and has been on Advisory Boards for Astra Zeneca and Glaxo Smith Kline. STH is a consultant for
Novartis, Synairgen, Merck, Wyeth and Centocor. STH has received lecture fees from their companies. RP is a consultant for Cardiovascular Therapeutics, Duska Therapeutics and NeuroSearch, and has received lecture fees from Merck and Novartis.

CONTRIBUTORS

STH, DED, AJC, JM and KSB contributed to the concept and design of the study. JM, AJC, RP, DED and STH contributed to the analysis and interpretation of data. STH was principal investigator; DED, RD, JM and AJC were co-investigators. AJC, JM, RP and STH drafted the article and revised it critically for intellectual content. JM and AJC conducted and supervised the study at the clinical sites.
REFERENCES


Figure Legends

Figure 1.
The plan of study at screening, baseline enrolment, during 12 weeks of etanercept treatment or placebo and subsequent follow up.

Figure 2.
The plan of study at screening, baseline enrolment, during 12 weeks of etanercept treatment or placebo and subsequent follow up.

Figure 3 (a) to (d).
The differences between groups for change in AQLQ, PC20, CRP and albumin from baseline after 12 weeks treatment with etanercept or placebo. The red bars represent means.

* One subject in the etanercept group failed to achieve a 20% fall in FEV$_1$ at the highest concentration of methacholine and a conservative estimate was obtained by calculating the cumulative PC20 on the next doubling concentration beyond the highest administered (i.e. 32 mg/ml).

Figure 4 (a) and (b).
The changes in (a) ACQ scores and (b) mean daily PEF (L/min) over the 12 week study period.
Figure 1
The plan of study at screening, baseline enrolment, during 12 weeks of etanercept treatment or placebo and subsequent follow up.

<table>
<thead>
<tr>
<th>STUDY WEEK</th>
<th>Run In Phase</th>
<th>Treatment Phase</th>
<th>Follow-up Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-2</td>
<td>-1 0 1 2 3 4 5 6 7 8 9 10 11 12</td>
<td>13 14 15 16</td>
</tr>
</tbody>
</table>

**SCREENING**
- Informed consent
- History
- Physical Exam
- Haematology
- Biochemistry
- Urinalysis
- Electrocardiography
- Chest x-ray
- Tuberculin test
- Hepatitis C test
- Pregnancy test
- Skin tests
- Lung Function (FEV₁, FVC)
- Twice daily PEF

**BASELINE**
- (pre-treatment)
- Physical Exam
- Vital signs
- Adverse events
- Lung Function (FEV₁, FVC)
- Twice daily PEF
- Sputum Analyses
- Methacholine challenge
- AQLQ
- ACQ

**DURING TREATMENT**
- Vital signs recorded
- Adverse events recorded
- Lung Function (FEV₁, FVC)
- Twice daily PEF
- ACQ

**POST TREATMENT**
- Physical Exam
- Vital signs
- Adverse events
- Haematology
- Biochemistry
- Urinalysis
- Electrocardiography
- Skin tests
- Lung Function (FEV₁, FVC)
- Twice daily PEF
- Sputum Analyses
- Methacholine challenge
- AQLQ
- ACQ

**FOLLOW UP**
- Physical Exam
- Vital signs
- Adverse events
- Pregnancy test
- Lung Function (FEV₁, FVC)
- Sputum Analyses
- Methacholine challenge
Patient Flow Diagram

Assessed for eligibility (59)

Randomised (39)

Excluded (20): Withdrawn, Not meeting Inclusion Criteria

Completed (37)

2 patient still to complete

Assumed Treatment Cluster (36)

Treatment 0 (18) (Placebo)

Treatment 1 (18) (Etanercept)
AQLQ Score (p=0.084) PC20 (p=0.234) *

CRP (p=0.029) Albumin (p=0.022)
Figures 4 (a) and (b)

The changes in (a) ACQ scores and (b) mean daily PEF (L/min) over the 12 week study period.
APPENDIX (AS AN ONLINE SUPPLEMENT)

Sample Size

Sample sizes were calculated with 80% power and an alpha value of 0.05 from two sample comparisons of means, based on the expected differences in ACQ and methacholine PC20 from our previous open-labelled study, and anticipated differences in the AQLQ based on the study by Berry et al. Recruitment of 32 subjects would have provided a sufficient sample size to detect differences in the primary endpoints; addition of a further 6 subjects allowing for a possible 20% drop-out rate.

Measurement of Bronchial Hyperresponsiveness

Subjects were asked not to take their short acting β2-adrenoceptor agonist for at least 4 hours prior to the bronchial provocation. Nebulised normal saline was first administered followed by isotonic 0.03 mg/ml methacholine (Sigma Co, Poole, Dorset, UK) through a dosimeter (Spira, Electro2, Spira, Finland) in doubling dilutions up to a maximum of 16 mg/ml until the FEV₁ (measured at intervals of up to five minutes) dropped by at least 20% of the baseline value. BHR was expressed as the cumulative provocative concentration of methacholine that reduced the FEV₁ by 20% of baseline (PC20) as determined by linear interpolation on a log scale. The procedure was discontinued if there was a fall in PEF of ≥15% after saline, or if there were any troublesome symptoms. Patients whose FEV₁ decreased by more than 15% after the inhalation of normal saline were arbitrarily assigned a PC20 value of 0.01 mg/ml.

Asthma Quality of Life and Control Questionnaires

The disease-specific AQLQ was assessed at baseline and at the end of the treatment phase. The adult version of this validated questionnaire comprises 32 questions based on four
categories including activity limitations, emotions, symptoms and exposure to environmental stimuli. The patients answered each question on a 7-point scale according to the level of impairment in the preceding 2 weeks, from 1 (extremely impaired) to 7 (no impairment); lower AQLQ scores indicating increased impairment. The ACQ was completed at each weekly visit. It consists of composite questions on asthma symptoms, including waking at night, waking with symptoms in the morning, shortness of breath, wheeze, limitation in activities, and bronchodilator use. Scores range from 0 to 6, with higher scores indicating more symptoms and reduced control of asthma. The questionnaire also includes measurement of FEV$_1$, which was also measured independently as part of the trial.

Sputum and Serum Biomarkers of Inflammation

Sputum processing involved adding 4 times an equal amount of 0.01M dithioerythritol (DTE) and phosphate buffered saline (PBS), filtered through a 70 µm filter and centrifugation for 10 mins at 400g at 4°C and the supernatants stored at -80°C. The serum and sputum supernatants were analysed for TNF-α using a Quantikine® high sensitivity (HS) TNF-α ELISA (R&D Systems, Abingdon, UK). IL-6, IL-1β and IL-8 levels were measured using Duoset® ELISA kits (R&D Systems, Abingdon, UK). A cytokine bead array (CBA) (CBA; BD Biosciences, USA) was also used to measure the inflammatory cytokines in both serum and sputum. Levels of CRP and albumin were measured by standard assays as part of the routine testing performed on blood samples taken from the subjects in the laboratories of Southampton General Hospital.
Adverse Events

The table shows the number of patients suffering with minor adverse events during the study.

There were no serious adverse events.

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Placebo</th>
<th>Etanercept</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Asthma exacerbations</td>
<td>36</td>
<td>36</td>
<td>0.720</td>
</tr>
<tr>
<td>Injection site rashes/bruises</td>
<td>0</td>
<td>5</td>
<td>0.014</td>
</tr>
<tr>
<td>Skin rashes</td>
<td>0</td>
<td>4</td>
<td>0.030</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>0</td>
<td>2</td>
<td>0.136</td>
</tr>
<tr>
<td>Lymphadenitis</td>
<td>1</td>
<td>0</td>
<td>0.324</td>
</tr>
<tr>
<td>Numbness to digits</td>
<td>0</td>
<td>1</td>
<td>0.298</td>
</tr>
<tr>
<td>Headaches</td>
<td>1</td>
<td>3</td>
<td>0.267</td>
</tr>
<tr>
<td>Somnolence</td>
<td>1</td>
<td>0</td>
<td>0.298</td>
</tr>
</tbody>
</table>

* Total number of exacerbations of asthma ± upper respiratory tract infections. Other indices refer to numbers of patients suffering the events. Significance values relate to Chi-square 2x2 tables with 19 patients receiving etanercept and 20 patients receiving placebo.

Sub-Group (Post-Hoc) Analyses

Multiple independent analyses of variance were used to explore relationships between baseline measurements (age, atopy, gender, medication, and sputum and serum biomarkers) with clinical outcomes, and there were no significant or consistent relationships present.

Ten patients prescribed anti-depressant medication at the start of the study were compared to 29 patients not prescribed them (Table 3). The mean reductions in ACQ and improvements in AQLQ scores between placebo and etanercept in the ten patients on antidepressants were non significant: -1.06 (95%CI -2.48, 0.36) and -0.66 (95%CI -1.54, 0.23), p=0.525, and 1.45 (95%CI 0.64, 3.54) and 1.03 (95%CI -0.18, 2.24), p=0.643 respectively. In those 29 patients not on antidepressant therapy however, mean reductions in ACQ and improvements in AQLQ scores between placebo and etanercept were however significant: -0.34 (95%CI -0.81,
0.13) and -1.27 (95% CI -1.68, -0.37), p=0.003, and 0.43 (95% CI -0.06, 0.91) and 1.01 (95% CI 0.58, 1.43), p=0.044 respectively.
Table 3

Changes in AQLQ and ACQ score from baseline after 12 weeks treatment or placebo, between patients on or not on antidepressant medication at recruitment into the study.

<table>
<thead>
<tr>
<th>CLINICAL OUTCOMES</th>
<th>PLACEBO</th>
<th>ETANERCEPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Before</td>
</tr>
<tr>
<td>ON antidepressants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AQLQ Score</td>
<td>5</td>
<td>3.44</td>
</tr>
<tr>
<td>ACQ Score</td>
<td>5</td>
<td>2.89</td>
</tr>
<tr>
<td>NOT on antidepressants</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>