Effect of antibiotic treatment on inflammatory markers and lung function in cystic fibrosis patients with *Pseudomonas cepacia*

D Peckham, S Crouch, H Humphreys, B Lobo, A Tse, A J Knox

Abstract

Background—The acquisition of *Pseudomonas cepacia* in patients with cystic fibrosis is associated with increasing deterioration in lung function and more frequent hospital admissions. *Pseudomonas cepacia* is usually resistant to several antibiotics in vitro, but the response of patients colonised with the organism has not been extensively studied in vivo.

Methods—A three month prospective study was performed to investigate the response of 14 *Ps cepacia* positive patients and 10 *Ps cepacia* negative patients to a two week course of intravenous antibiotics. All those who were *Ps cepacia* negative and six of the 14 *Ps cepacia* positive patients had *Ps aeruginosa* in their sputum which was sensitive to the prescribed therapy. The inflammatory markers C-reactive protein, white blood cell count, serum lactoferrin, neutrophil elastase, alpha-1-antitrypsin complex, and tumour necrosis factor alpha (TNFca), C-reactive protein, and white blood cell count have been shown previously to reflect the inflammatory process in patients with cystic fibrosis and are reduced following effective antipseudomonal treatment in patients colonised with *Ps aeruginosa*.2–10 It is not known, however, whether similar changes are seen after treatment in patients colonised with *Ps cepacia*. We have therefore prospectively studied the effectiveness of intravenous antibiotic therapy in a group of adult patients with cystic fibrosis, with and without *Ps cepacia*, measuring treatment response as change in inflammatory markers, lung function, and body weight.

Methods

STUDY DESIGN

This was a prospective study which included all patients who required two weeks of intravenous antibiotics for infective exacerbations of cystic fibrosis over a three month period at Nottingham City Hospital. Patients were assessed both before and at the end of the two week course of antibiotics with measurements of body weight, lung functions, and venous blood samples for measurements of inflammatory markers.

PATIENTS

Twenty four adult patients were studied, 10 of whom were colonised with *Ps aeruginosa* without *Ps cepacia* (*Ps cepacia* negative) and 14 of whom were colonised with *Ps cepacia*, with or without *Ps aeruginosa* (*Ps cepacia* positive). Clinical details are outlined in table 1. All patients had been chronically infected with *Ps aeruginosa* for more than two years. Of the patients who were *Ps cepacia* positive, this bacterium had been isolated from one patient three weeks before antibiotic therapy while the remaining 13 had repeatedly been positive for...
Ps cepacia for more than six weeks. Two patients who were Ps cepacia positive and one who was Ps cepacia negative were on low dose oral prednisolone prior to antibiotic therapy (10–20 mg/day). None of the patients were started on steroids over the three month study period. Four patients in both groups received their antibiotics at home while being reviewed weekly on the ward and the remainder were treated as inpatients. Pathogens isolated from sputum before the start of intravenous antibiotic therapy are summarised in table 2.

LUNG FUNCTION

Forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) were measured as the highest of three blows on a Vitalograph spirometer (Buckingham, UK).

MICROBIOLOGY

Neat sputum and a 1:10 000 dilution of sputum from patients with cystic fibrosis was routinely cultured for Haemophilus influenzae, Staphylococcus aureus, and Ps aeruginosa following digestion with N-acetyl cysteine using cellobulin chocolate agar and MacConkey agar. Investigation for the presence of mycobacteria and atypical respiratory pathogens was carried out when indicated. Sputum samples were also inoculated on to Ps cepacia selective agar medium (MAST, UK), incorporating ticarcillin (100 mg/l) and polymyxin B (30 000 units/l). Agar plates were incubated for 40 hours at 37°C and Ps cepacia was identified by colonial appearance, oxidase reaction, biochemical reaction, and resistance to polymyxin B. Antimicrobial susceptibility testing to gentamicin, tobramycin, ceftazidime, azlocillin, ciprofloxacin, aztreonam, imipenem, and polymyxin B was carried out using the disc diffusion method.

ANTIBIOTIC TREATMENT

All patients who were Ps cepacia negative received an aminoglycoside (gentamicin or tobramycin) with 5 g three times daily azlocillin (one patient), or 2 g three times daily ceftazidime (seven patients), 2 g three times daily aztreonam (one patient) or 1 g three times daily imipenem alone (one patient). Patients who were Ps cepacia positive received a combination of an aminoglycoside with either ceftazidime (eight patients), azlocillin (five patients), or aztreonam (one patient) at the identical doses to patients who were Ps cepacia negative. Antibiotic regimens were selected according to sensitivity results of both Ps aeruginosa and Ps cepacia, and in patients who were Ps cepacia positive combination therapy was used. The choice of antibiotics was arbitrary when a multiresistant strain of Ps cepacia was isolated. In the absence of Ps aeruginosa. At the start of treatment all 10 isolates of Ps aeruginosa among patients who were Ps cepacia negative and five isolates of Ps aeruginosa from patients who were Ps cepacia positive were fully sensitive to the antibiotics used. One patient who was Ps cepacia positive grew a Ps aeruginosa isolate which proved to be sensitive to tobramycin alone. Of the 14 patients who were Ps cepacia positive 12 had a multiresistant strain of Ps cepacia in their sputum while the isolates from two patients were sensitive to ceftazidime alone. Two patients in the Ps cepacia positive group and one in the Ps cepacia negative group received either high dose oral amoxicillin (3 g twice daily) or intravenous cefuroxime (1.5 g three times daily) to treat additional H influenzae (table 2), while one patient in each group was treated with oral flucloxacinil (1 g four times daily) for additional Staphylococcus aureus infection. Serum aminoglycoside levels were measured around the fourth dose and again on the third or fourth day thereafter, with dose adjustment as appropriate to maintain a serum peak concentration of 7–10 mg/l. Five patients who were Ps cepacia negative and five who were Ps cepacia positive were on long term nebulised antibiotic therapy which was discontinued during the study period. Of the patients who were Ps cepacia negative two were on colomycin (1 meganuit twice daily), two on gentamicin (80 mg twice daily) and one on tobramycin (80 mg twice daily), while amongst patients who were Ps cepacia positive four were on colomycin (1 meganuit twice daily) and one was on gentamicin (80 mg twice daily).

INFLAMMATORY MARKERS

Full blood count with differential counts was measured by conventional automated analysis, C-reactive protein by a nephelometry method, and other inflammatory markers by enzyme linked immunosorbent assay (ELISA) following serum storage at −70°C. The lactoferrin ELISA used had a lower detection limit of 0.005 nmol/l. Immunoreactive TNF was measured according to a previously described method with a lower detection limit of 6.25 pg/ml. Elastase/α1-antiprotease was measured using a modification of a previously described method where the streptavidin horseradish peroxidase step was replaced with...
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### Table 3 Median (range) results for inflammatory markers before and after treatment in the combined group of 24 adult patients with cystic fibrosis

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>White cell count (× 10^9/l)</td>
<td>10.9 (2.4–21.5)</td>
<td>8.7 (2.0–18.3)</td>
<td>0.005</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>29 (&lt;11–169)</td>
<td>&lt;11 mg/l (&lt;11–130)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lactoferrin (nmol/l)</td>
<td>6.97 (3.8–8.99)</td>
<td>4.97 (3.29–7.87)</td>
<td>0.02</td>
</tr>
<tr>
<td>Tumour necrosis factor (pg/ml)</td>
<td>42.25 (20–1055)</td>
<td>27.2 (6–604.5)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Elastase/α1-antitrypsin complex (ng/ml)</td>
<td>728 (277–1541)</td>
<td>407.5 (54–842)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

There was no difference between the % improvement in both FEV1 and FVC among the six patients who were *Ps aeruginosa* and *Ps cepacia* positive, and the six patients who were *Ps cepacia* positive but *Ps aeruginosa* negative.

### SPIROMETRY

When patients who were *Ps cepacia* positive and *Ps cepacia* negative were combined, lung function improved significantly following antibiotic treatment compared with baseline (table 4).

<table>
<thead>
<tr>
<th>Inflammatory markers following treatment</th>
<th><em>Ps cepacia</em> positive</th>
<th><em>Ps cepacia</em> negative</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>White cell count (× 10^9/l)</td>
<td>1.1 (2.9–10.2)</td>
<td>1.6 (1.2–8.0)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>32 (14.7–77)</td>
<td>14 (21–74)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Lactoferrin (nmol/l)</td>
<td>0.63 (0.93–1.1)</td>
<td>0.12 (0.80–0.98)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Tumour necrosis factor (pg/ml)</td>
<td>38.75 (33–235)</td>
<td>0.2 (106.6–450.5)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Elastase/α1-antitrypsin complex (ng/ml)</td>
<td>950 (868–1552)</td>
<td>807 (100–836)</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>

The p value shown is for the difference in the change in each variable between patients who were *Ps cepacia* positive and those who were *Ps cepacia* negative.
When serum lactoferrin levels are corrected for the number of circulating neutrophils the median (range) for the 24 patients before treatment was 0.885 (0.503–1.479) nmol/10^6 neutrophils and 0.947 (0.368–2.636) nmol/10^6 neutrophils following treatment (p > 0.05). There were no significant differences between corrected values of serum lactoferrin and TNF levels before and after treatment when patients who were Ps cepacia positive and negative were compared (p > 0.05).

Discussion

Pseudomonas cepacia has been isolated with increasing frequency in specialist centres within the UK. Isolation of Ps cepacia from patients with cystic fibrosis is associated with poor lung function, increasing age, recent hospitalisation, and close hospital or social contact with other patients who are Ps cepacia positive and siblings. Despite some evidence to suggest that colonisation with Ps cepacia heralds a poorer prognosis, it is unclear whether this is because the organism is pathogenic or because it is a marker for increased disease severity due to other factors. Pseudomonas cepacia is non-pathogenic in healthy individuals and in animal models it has been found to be relatively avirulent compared with Ps aeruginosa. It is possible that Ps cepacia acts synergistically with other bacteria to exacerbate chest disease in cystic fibrosis.

In this study we have investigated the effect of intravenous antipseudomonal antibiotic treatment in patients with Ps cepacia infection and have compared this with patients colonised by Ps aeruginosa. Several parameters were used to assess response including lung function and various inflammatory markers. In our group of 24 patients studied prospectively we found that lung function and Schwachman scores were lower in patients colonised with Ps cepacia than in those with Ps aeruginosa alone. This is consistent with the results of other studies and suggests an association between colonisation with Ps cepacia and poor clinical status. Despite the fact that patients who are Ps cepacia positive had worse lung function and Schwachman scores, antibiotic treatment was equally effective in improving lung function and weight in both groups. Both these parameters improved significantly over the two weeks of treatment in both groups of patients and was similar to that reported in previous studies of patients colonised with Ps aeruginosa. The fact that weight improved equally in patients who were Ps cepacia positive despite their generally poorer clinical status may partially reflect the fact that more of them were on nasogastric nutritional supplementation.

Several inflammatory markers were measured including white blood cell count and serum levels of C-reactive protein, lactoferrin, neutrophil elastase/α1-antitrypsin complex, and TNF. These have all been shown previously to reflect the inflammatory process in patients with cystic fibrosis. Previous studies of patients colonised with Ps aeruginosa have shown that values of these markers fall following treatment with intravenous antipseudomonal antibiotics.

The inflammatory markers elastase/α1-antitrypsin, lactoferrin, and C-reactive protein fell in response to intravenous antibiotic therapy in both patients who were Ps cepacia positive and those who were Ps cepacia negative. The changes seen were similar to those found previously in studies of patients colonised with Ps aeruginosa. No significant change in TNF levels occurred following antibiotic therapy in either group, but many patients had undetectable levels of TNF both before and after treatment. Previous studies have expressed lactoferrin levels as absolute values. When we did the same we found a significant reduction in lactoferrin levels with antibiotic treatment. The sole source of lactoferrin is the neutrophil, however, and neutrophil values also fell. When the lactoferrin level was adjusted for the neutrophil count no significant change was seen. This suggests that the fall in lactoferrin levels is largely due to the fall in neutrophil count. As with the lung function results, there was no significant difference in improvement in any of the inflammatory markers between patients who were Ps cepacia positive and those who were Ps cepacia negative.

The good clinical response to intravenous antibiotic therapy among patients who were Ps cepacia positive is surprising in the light of the in vitro sensitivity pattern seen with Ps cepacia. The organism is resistant to most commonly used antipseudomonal agents, but combinations of antibiotics – for example, aminoglycosides such as gentamicin and a β-lactam agent such as azlocillin – may, however, inhibit the growth of Ps cepacia synergistically. In vitro combinations of other antibiotics have been shown to be synergistic against Ps cepacia. Synergy between two antibiotics (rifampicin, imipenem, and ciprofloxacin) has previously been demonstrated and therefore two or more antimicrobial agents may be needed. Many patients with cystic fibrosis who are colonised with Ps cepacia also carry Ps aeruginosa and exacerbation may be caused by Ps aeruginosa with or without H influenzae or Staph aureus. Our clinical experience would seem to indicate that combination chemotherapy results in vivo activity against Ps cepacia despite resistance in vitro. Nottingham isolates of Ps cepacia are resistant to the aminoglycosides, azlocillin, ciprofloxacin, imipenem, aztreonam, and polymyxin B, but some are moderately sensitive to ceftazidime and most adult patients with cystic fibrosis show the same strain (unpublished observation).

We have considered several explanations for the clinical improvement of patients who were Ps cepacia positive despite in vitro antimicrobial resistance. Firstly, it is possible that in vitro antibiotic sensitivities do not reflect the in vivo situation within the lung due to the milieu of the inflammatory response. Alternatively the in vivo response may be due to synergism between antibiotics. Current methodology used in antibiotic susceptibility testing of Ps cepacia may be less appropriate for this organism which is slower growing than Ps aeruginosa and grows
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preferentially at 30°C. It is also possible that a heavy growth of Ps cepacia may inhibit the recognition of other organisms such as Staph aureus and Haemophilus. Whilst this is a possibility it would not explain the results of treatment directed towards Ps aeruginosa in our study. An alternative explanation is that the response in both patient groups is due solely to effective physiotherapy which is causing improvement by enhancing sputum expectoration and reducing the inflammatory stimulus, thus reducing levels of inflammatory markers. This latter possibility would seem unlikely as previous studies have shown that intravenous antibiotics alone are more effective than physiotherapy alone at reducing Ps aeruginosa counts in sputum.20 Antibiotics are also known to have other effects which may modify the response to infection including the release of endotoxin and the inhibition of the cytokine cascade.26 The possible immunomodulatory effect combined with some in vivo antibacterial activity, physiotherapy, and nutritional support may explain the clinical improvement. Consequently, beneficial effects might be explained despite persistence of the organism. Lastly, we considered whether the response to treatment in patients colonised with Ps cepacia may reflect treatment of Ps aeruginosa rather than Ps cepacia. For this reason we have compared the two subgroups of patients who were Ps cepacia positive (with and without Ps aeruginosa) and have found no differences in either lung function or inflammatory markers between patients colonised with Ps aeruginosa and those colonised with Ps cepacia. This suggests that treatment was as effective when only Ps cepacia was present. Alternatively, the clinical response to antibiotic treatment among patients who appeared to be colonised by Ps cepacia alone may simply reflect the treatment of underlying Ps aeruginosa as the inability to isolate this organism from the sputum does not exclude its presence within the lower respiratory tract. The fact that we found that patients who were Ps cepacia positive responded well to antibiotics suggests that the reason for the greater decline in their lung function in some studies may be the result of lung damage occurring between antibiotic courses.2 It is interesting that our patients who were Ps cepacia positive required more courses of intravenous antibiotics over the previous 12 months than those patients who were Ps cepacia negative.

We conclude, therefore, that antibiotic treatment of patients who are Ps cepacia positive is often effective in vivo despite the multiresistant nature of this organism in vitro. Further work is required to determine the relation between in vitro sensitivity results and the response in vivo and the pattern of inflammatory response over a prolonged period in patients who are Ps cepacia positive. From a practical point of view antibiotic should not be withheld from patients because of in vitro resistant patterns.

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