Original Articles

Phase 1 safety trial of Filgrastim (r-metHuG-CSF) in non-neutropenic patients with severe community-acquired pneumonia

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The objectives of the present study were to: (1) evaluate the safety of Filgrastim therapy in non-neutropenic patients with severe community-acquired pneumonia; (2) determine the absolute neutrophil count (ANC) response to various dosages of Filgrastim in non-neutropenic patients with active infection; and (3) describe the impact of therapy with Filgrastim in combination with antibiotics on selected pneumonia-related clinical parameters.

The study design was an open-label, dose-ranging, clinical trial, set in the General Clinical Research Unit of a large, public community hospital. The study population consisted of 30 patients who had presented to the Emergency Department with severe, community-acquired pneumonia.

One of five dosages (75, 150, 300, 450 or 600 µg day⁻¹) of Filgrastim (r-metHuG-CSF) was given subcutaneously daily for 10 days, until discharge or until the absolute neutrophil count >75 × 10⁹ l⁻¹, whichever was earlier. Vital signs, pulse oximetry, arterial blood gases, daily complete blood counts with differential, serum chemistries, coagulation profiles, electrocardiograms, chest radiographs, plasma G-CSF concentrations and duration of hospitalization were measured.

There was no evidence of Filgrastim-related lung injury or evidence of extra-pulmonary toxicity. There was no apparent dose-response effect of Filgrastim on pneumonia-related clinical variables. Dosages of Filgrastim between 150 and 600 µg day⁻¹ had similar effects on increasing the ANC.

Filgrastim appeared to be safe in non-neutropenic patients with severe, community-acquired pneumonia when given in dosages of 75–600 µg day⁻¹ in combination with appropriate antibiotic therapy. Further study is needed to determine the effect of Filgrastim on morbidity, mortality and duration of symptoms in this patient population.

Introduction

In spite of advances in antibiotic therapy and improved life-support measures, pneumonia and influenza remain the sixth leading cause of death in the United States, and the most common cause of death from infectious disease (1). The mortality of patients who require hospitalization for pneumonia typically ranges from 10 to 25%. However, in the subset of patients who require admission to an intensive care unit (ICU), mortality rates have been reported to range from 25 to 93% (2-4). Most patients who die have well-recognized co-morbid risk factors, such as advanced age, chronic lung disease, diabetes or alcoholism.

While the pathogenesis of bacterial pneumonia is not fully understood, it is thought to result from colonization of the posterior pharynx with pathogenic
bacteria and subsequent micro-aspiration of these bacteria into the lower respiratory tract. If host respiratory-defence mechanisms, such as cough, mucociliary elevator action or phagocytic function, are inadequate, if the inoculum is large, or if the bacteria are particularly virulent, pneumonia may result.

Granulocyte colony-stimulating factor (G-CSF) is a growth factor produced by monocytes, fibroblasts and endothelial cells. It has at least three separate roles in vitro: a direct influence upon proliferation and development of neutrophil progenitor cells (5-8); stimulation or proliferation and functional activation of more mature cells of the neutrophil lineage (9,10); and ability to synergize with other haematopoietic growth factors (11,12). Non-glycosylated, recombinant human G-CSF (rHuG-CSF, Filgrastim) has been cloned and expressed, and has been approved by the Food and Drug Administration (FDA) (U.S.A.) and other regulatory agencies to decrease the incidence and duration of severe neutropenia induced by cytotoxic chemotherapy for solid tumours (13-16), and for conditioning regimens for autologous bone marrow transplantation in patients with non-myeloid malignancies (17). Filgrastim is also approved for the treatment of severe chronic neutropenia (18) and for haematopoietic reconstitution following bone marrow transplantation (19). It has been shown to reduce the incidence of fever associated with neutropenia, the incidence of culture-confirmed infections, the total number of days of treatment with intravenous antibiotics, and the total number of days of hospitalization (20-22).

In non-neutropenic animal models of pneumonia, treatment with Filgrastim improved bacterial clearance from the lung and reduced mortality (23). In a mouse model, Filgrastim administered from 24 h before bacterial challenge to 3 days after challenge was found to improve survival in splenectomized mice but not in sham-operated mice exposed to Streptococcus pneumoniae (24). A recently published study examined the use of Filgrastim, given with antibiotics, on morbidity and mortality in animals with pneumonia and sepsis (25). Rabbits were infected with penicillin-sensitive, Gram-negative bacteria and then randomized to receive penicillin with placebo or 6.5-8 \( \mu \)g kg\(^{-1}\) day\(^{-1}\) Filgrastim for 5 days. The rabbits treated with Filgrastim had a significant increase in the number of neutrophils by Day 4 of the study compared with rabbits treated with placebo, and there was a trend towards improved survival in the Filgrastim-treated animals, with most of the survival benefit seen in the first 24 h of treatment.

The present dose-ranging study was designed to evaluate the safety of Filgrastim therapy in non-neutropenic patients with severe community-acquired pneumonia. As neutrophils had been reported to play a potential role in the pathogenesis of the acute respiratory distress syndrome (ARDS) (26), the potential of Filgrastim-induced neutrophilia to aggravate pulmonary inflammation and injury was of primary interest. Secondary objectives were to determine the absolute neutrophil count (ANC) response to various dosages of Filgrastim in non-neutropenic patients with active infection, and to describe the impact of therapy with Filgrastim in combination with antibiotics on selected pneumonia-related clinical parameters.

### Methods

**PATIENTS**

The study was approved by the Institutional Review Board of Louisiana State University Medical Center and was conducted in the General Clinical Research Center of Charity Hospital of Louisiana. Community-acquired pneumonia was defined by the presence of a new productive cough or changes in a chronic cough, core body temperature \( \geq 38.3^\circ \text{C} \) or \( <36^\circ \text{C} \), and a new infiltrate on chest radiography that is compatible with the diagnosis of pneumonia. Patients who required hospitalization for the treatment of community-acquired pneumonia were eligible to be enrolled in this trial if two or more risk factors for adverse outcome (Table 1) were present. Patients were excluded from the trial if any of the exclusion criteria (Table 2) were present. Written informed consent was obtained from all patients enrolled.

### STUDY DESIGN

Sputum and blood cultures were obtained prior to the administration of cefuroxime 1.5 g intravenously

### Table 1. Risk factors for severe disease

<table>
<thead>
<tr>
<th>Risk Factor</th>
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<tbody>
<tr>
<td>Age 60 years</td>
</tr>
<tr>
<td>Pre-existing chronic illness, such as COPD, diabetes mellitus or alcoholism</td>
</tr>
<tr>
<td>Respiration rate ( &gt;25 ) and (&lt;40 \text{ breaths min}^{-1} )</td>
</tr>
<tr>
<td>Hypoxia ( (\text{PaO}_2 \text{ on room air} &lt; 65 \text{ mmHg}) )</td>
</tr>
<tr>
<td>Relative leukopenia ( (\text{WBC} 3.6 \times 10^9 \text{ l}^{-1}) )</td>
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<tr>
<td>Blood urea nitrogen ( &gt;20 \text{ mg dl}^{-1} ) in the absence of chronic renal disease</td>
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<tr>
<td>Splenectomy or prior splenic radiation</td>
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<tr>
<td>Chronic steroid therapy</td>
</tr>
<tr>
<td>Multiorganic infiltrates on chest radiograph</td>
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</tbody>
</table>

COPD, chronic obstructive pulmonary disease.
TABLE 2. Exclusion criteria

| Age <18 years                  |
| Pregnant                      |
| Allergy to penicillin, cephalosporins or *Escherchia coli*-derived products |
| Need for >50% supplemental oxygen, need for ventilatory assistance upon admission, or diagnosis of ARDS |
| Suspected tuberculosis, post-obstructive pneumonia or non-bacterial pneumonia |
| Haematologic abnormality (platelet count <10 x 10^9 l^-1, ANC <1.5 x 10^9 l^-1 or >40 x 10^9 l^-1, or any haematologic malignancy) |
| Cardiac abnormalities (uncontrolled hypertension with diastolic blood pressure >115 mmHg, symptomatic cardiac arrhythmias, unstable angina, or NYHA Class IV congestive heart failure) |
| Shock, acute hepatic or renal failure, disseminated intravascular coagulation (DIC), or respiratory failure (Pao2/Fio2 <200) |
| Established diagnosis of HIV infection |
| Previous treatment with cytotoxic chemotherapy or radiotherapy |

ARDS, adult respiratory distress syndrome; ANC, absolute neutrophil count.

(i.v.) every 8 h for at least 48 h. Groups of five to nine patients were given one of five dosages of Filgrastim (75, 150, 300, 450 or 600 μg day^-1) subcutaneously (SC) for a maximum of 10 days or until an ANC >75 x 10^9 l^-1, if earlier. The first dose of Filgrastim was given on the day of hospitalization regardless of the time, but subsequent doses were given daily at 9 am. Antibiotics were switched to cefuroxime 250 mg orally (p.o.) twice daily if all the following criteria were met: core body temperature ≤37.8°C, respiratory rate <20 min^-1, chest radiograph stable or improved, and ability to tolerate oral medication. No antipyretics were given but propoxyphene was allowed for analgesia.

LABORATORY TESTS AND CLINICAL OBSERVATIONS

Throughout the study observation, patients were monitored with vital signs every 4 h and daily symptom assessments. Continuous pulse oximetry was performed for the first 72 h. Arterial blood gases were obtained at baseline and then as indicated. Severity of illness was measured at baseline and intermittently using the APACHE II scoring system. Daily complete blood counts with ANC count were obtained before administration of Filgrastim and serum chemistries and coagulation profiles were drawn weekly. Chest radiographs were obtained every other day and evaluated by a staff pulmonologist for changes from baseline values. At baseline and every morning before administration of Filgrastim, a blood sample was drawn in an ethylenediaminetetraacetic acid (EDTA)-coated vacuum tube for determination of plasma G-CSF concentration. These blood samples were centrifuged, frozen in cryovials at -70°C, and shipped to Amgen (Thousand Oaks, CA, U.S.A.) for analysis. Electrocardiograms (ECGs) were obtained at baseline and then periodically thereafter as clinically relevant.

The duration of hospitalization was defined as the time from admission until the time that discharge eligibility criteria were met. For protocol purposes, a patient was considered eligible for hospital discharge once his or her constitutional symptoms were mild or resolved, he or she could tolerate oral diet and antibiotics, core body temperature was <37.8°C for 48 h, and there was no growth on follow-up blood cultures if bacteraemic. Outpatient follow-up exams and chest radiographs were obtained 2 weeks and 4 weeks after discharge.

ADVERSE EVENTS

All adverse events occurring during the 2-week period following presentation were noted for severity and relationship to study drug according to the World Health Organization (WHO) adverse events standard grading score (27).

STUDY DRUG

The rHuG-CSF used in this study was Filgrastim (NEUPOGEN®), a non-glycosylated product produced by recombinant DNA techniques using *E. coli*. The Filgrastim was supplied by the manufacturer (Amgen Inc., Thousand Oaks, CA, U.S.A.).
### TABLE 3. Demographic Data of Study Patients

<table>
<thead>
<tr>
<th>Dosage (μg day⁻¹)</th>
<th>Age Median (range)</th>
<th>Sex M/F</th>
<th>Apache II Score Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>40 (26–73)</td>
<td>5/1</td>
<td>10 (8–18)</td>
</tr>
<tr>
<td>150</td>
<td>41 (35–53)</td>
<td>4/1</td>
<td>8 (5–13)</td>
</tr>
<tr>
<td>300</td>
<td>49 (30–65)</td>
<td>7/2</td>
<td>9 (6–22)</td>
</tr>
<tr>
<td>450</td>
<td>42 (34–62)</td>
<td>3/2</td>
<td>8 (4–14)</td>
</tr>
<tr>
<td>600</td>
<td>40 (20–49)</td>
<td>2/3</td>
<td>11 (6–13)</td>
</tr>
</tbody>
</table>

**STATISTICAL ANALYSIS**

Due to the small number of patients (n=30), the primary focus was only descriptive statistics of central tendency and dispersion categorized by dosage group and time were done. No inferential statistical analyses were done.

**Results**

**PATIENTS**

Thirty patients meeting the community-acquired pneumonia case definition were enrolled in the study. A summary of the demographic data of the treatment groups including median APACHE II scores is given in Table 3. Twenty-nine patients completed study-drug administration through Day 10 or hospital discharge. One patient was removed from the study on Day 3 when it was determined that his symptoms were not due to community-acquired pneumonia, but were cardiac in origin. Twenty-three of 29 patients (79%) completed one of two post-discharge follow-up visits, and 21 patients (72%) completed both. There were no on-study deaths. Twenty-one patients (70%) were evaluable for the assessment of efficacy of Filgrastim dosing and ANC response, and 30 patients were evaluable for safety analysis.

**LABORATORY TESTS AND CLINICAL OBSERVATIONS**

Five of the 30 patients were bacteraemic, three in the 75 μg day⁻¹ group and one each in the 300 and 600 μg day⁻¹ groups. Of the blood cultures from the bacteraemic patients, four grew S. pneumoniae and one grew Salmonella enteritidis. An additional seven patients had sputum cultures positive for a respiratory pathogen: two S. pneumoniae, four Hemophilus influenzae and one Staphylococcus aureus. One patient presented with a pleural effusion which was subsequently determined to be a polymicrobial empyema. Surgical decortication was required for resolution. The patient with S. enteritidis bacteraemia was determined to be HIV positive after enrollment. One patient was also diagnosed to have Legionella infection as diagnosed by a positive direct fluorescent antibody (DFA).

Oxygenation remained normal or improved in most patients, irrespective of Filgrastim dosage. The temporal relationship between the PaO₂/FiO₂ ratios and Filgrastim dosage is shown in Fig. 1. There was little variation in the median values of systolic and diastolic blood pressure, pulse rate and respiratory rate during the study. Median core body temperature decreased from approximately 39 to 37°C by hospital discharge.

The median duration for all patients for fever was 4 days; for i.v. antibiotic treatment, 6 days; for Filgrastim treatment, 7 days; and for hospitalization, 8 days. There were no clear patterns of dose response in these variables. Within each dosage group, the median duration of Filgrastim treatment differed from the median duration of i.v. antibiotic use and hospitalization by no more than 2 days (Fig. 2).

Figure 3 illustrates the median daily ANC for the five dosage groups. The 75 μg day⁻¹ dose had the smallest impact on the ANC, and a relationship between dosage and ANC and dosage and peak ANC was not established. The median percent change over baseline ANC values was 128, 209, 287, 309 and 194% for the 75, 150, 300, 450 and 600 μg day⁻¹ groups, respectively. The highest ANC response was \( 101 \times 10^9 \text{ l}^{-1} \) which occurred on Day 4, 1 day before
discharge, in a 53-year-old man in the 300 μg day⁻¹ group. This patient was severely ill on presentation with an APACHE II score of 22.

With the exception of differential leukocyte shifts, there was no apparent WHO toxicity shift of two or more grades in blood chemistries, haematology, coagulation or urinalysis. While receiving Filgrastim, 14 patients (47%) had increases in monocyte counts, 13 (43%) had increases in basophil counts, and 12 (40%) had increases in eosinophil counts that resulted in WHO toxicity shifts of two grades or more.

There was no relationship between Filgrastim dosage and the rate of radiographic resolution. The median time to resolution of the radiographic abnormalities was 12.5 days (range 3–22 days). Pneumonia-related symptoms (i.e. fever, cough, chest pain and malaise) improved in all patients during the study. Analyses of ECGs were unchanged from baseline and showed no relationship to Filgrastim dosage.

The median daily G-CSF plasma concentrations were independent of the dosage of Filgrastim (Fig. 4). Concentrations tended to decrease from the day of admission to nearly undetectable levels by discharge, regardless of continuation or cessation of Filgrastim.

There were three noteworthy protocol violations: three patients in the 150 μg day⁻¹ group received acetaminophen during the study, although this was prohibited by the protocol. All patients received at least two doses of Filgrastim and were evaluable for analyses of safety, but only 21 patients received >5 days of Filgrastim and were therefore appropriate for
analyses of the dose-response relationship between Filgrastim and ANC.

Discussion

Preclinical investigations of Filgrastim have repeatedly shown it to be effective in enhancing host defences against bacterial challenges in several non-neutropenic models of sepsis, pneumonia and soft-tissue infection (23,28). In patients, many of these infectious conditions are also risk factors for ARDS. The lung is normally one of the major sites of sequestration for non-circulating neutrophils (26), and neutrophils can be detected in large numbers in the interstitium and air spaces during biopsy as well as in the bronchoalveolar lavage fluid of patients with ARDS. As activated neutrophils have been implicated in the pathogenesis of microvascular injury in the lung, there has been concern regarding the potential of Filgrastim to potentiate lung inflammation (29). Filgrastim not only increases the number of neutrophils in the pulmonary capillaries but also upregulates the expression of the surface receptors CD11b and CD11c, and primes neutrophils for chemotaxis and oxygen-radical production (30,31).

Despite this concern, the reported incidence of ARDS in cancer patients receiving Filgrastim has only been 0.02%, and nearly all these cases have occurred in patients with underlying risk factors for ARDS, such as sepsis (Amgen, data on file). Furthermore, Kanazawa et al. (32) have shown that recombinant G-CSF pretreatment attenuated the lung injury induced by intravenous endotoxin in guinea pigs, and Koizumi et al. (33) have shown that recombinant G-CSF does not worsen endotoxin-induced lung injury in sheep. Fink et al. (34) have made a similar observation in pigs.

This current trial demonstrated the safety and feasibility of combining exogenous G-CSF therapy (Filgrastim) with antibiotics in the treatment of pneumonia in a group of patients with risk factors for ARDS. The degree of blood neutrophilia induced by Filgrastim therapy was well tolerated in the patients, and no signs or symptoms of pulmonary toxicity were observed, nor were there any cases of ARDS. In contrast, Terashima et al. (35) have shown that recombinant G-CSF can potentiate lung injury induced by intratracheal endotoxin in neutropenic guinea pigs. Similarly, King et al. (29) have shown that Filgrastim can potentiate experimental lung injury induced by alpha naphthylthiourea (ANTU) and hydrochloric acid (HCl).

Although pneumonia is the major predisposing factor to ARDS in 20–40% of cases (36), the apparent safety of Filgrastim in this high-risk population is conjectured to be related to beneficial effects on pulmonary host defences. Infection, and the host responses to infection, are major aetiological factors responsible for the induction and perpetuation of lung injury in ARDS and multisystem organ failure. The host response to infection may be more critical determinant of the outcome of sepsis and ARDS than the original inciting stimulus (37). If Filgrastim speeds bacterial clearance from the lung, then treated patients could, theoretically, have less pulmonary inflammation and a lower risk of ARDS than untreated patients.

Although all the patients in this study had risk factors for adverse outcomes, patients with evidence of organ failure at presentation were intentionally excluded because of concerns about differentiating between drug-related and disease-related adverse events. It remains possible that proliferation and activation of neutrophils by Filgrastim in a patient with pre-existing ARDS could potentiate lung injury, but the answer to this concern must await further clinical investigation.

Conclusions

Exogenous G-CSF administration, in the form of Filgrastim, appeared to be safe in non-neutropenic patients with severe community-acquired pneumonia when given in dosages of 150–600 μg day⁻¹ in combination with appropriate antibiotic therapy. There was no evidence of Filgrastim-related lung injury and no apparent dose-response effect of Filgrastim on pneumonia-related clinical variables. Dosages of Filgrastim between 150 and 600 μg day⁻¹ had similar effects on ANC. Further study is needed to determine the effect of Filgrastim on morbidity, mortality and duration of symptoms in patients with severe community-acquired pneumonia.

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