Adjunctive efficacy of granulocyte colony-stimulating factor on treatment of *Pseudomonas aeruginosa* pneumonia in neutropenic and non-neutropenic hosts

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Objectives: Granulocyte colony-stimulating factor (G-CSF) stimulates proliferation of neutrophils and enhances their phagocytic and microcidal activity. Increasing resistance to existing antibacterials and the dearth of new alternatives have complicated the treatment of Gram-negative infections. The aim of this study was to evaluate the efficacy of G-CSF in the treatment of *Pseudomonas aeruginosa* pneumonia when administered in combination with ceftazidime in both neutropenic and non-neutropenic hosts.

Methods: A group of mice were rendered neutropenic with cyclophosphamide. Pneumonia was induced by intratracheal instillation of \(\sim 5 \times 10^7\) cfu/mL and \(\sim 5 \times 10^9\) cfu/mL (LD\(_{100}\)) of the organism to neutropenic and non-neutropenic mice, respectively. Two hours after inoculation, the mice received normal saline and 5% dextrose, G-CSF (300 \(\mu\)g/kg per day \(\times\) 3 days), ceftazidime (2000 mg/kg \(\times\) 2 doses) or a combination of G-CSF and ceftazidime. Survival was monitored at different time points for 5 days.

Results: Treatment with G-CSF showed a dose-dependent increase in survival from 50 to 300 \(\mu\)g/kg. In neutropenic mice, survival was markedly better in the G-CSF + ceftazidime group compared with controls (\(P = 0.0001\)), G-CSF (\(P = 0.0002\)) or ceftazidime (\(P = 0.0172\)). In non-neutropenic mice, survival in the G-CSF + ceftazidime group (20%) was significantly higher than in the control and G-CSF groups (\(P = 0.0001\) but not significantly higher than ceftazidime alone (9%) (\(P > 0.05\)).

Conclusions: G-CSF administered in combination with antibiotic after onset of severe *P. aeruginosa* pneumonia may improve therapeutic outcome and this suggests a new treatment option in the management of pneumonia especially in neutropenic patients.

Keywords: G-CSF, immunomodulators, *P. aeruginosa*, ceftazidime

Introduction

Granulocyte colony-stimulating factor (G-CSF) enhances the phagocytic and bactericidal activities in both neutropenic and non-neutropenic hosts.\(^1,2\) The effects of G-CSF alone or as an adjunct to therapy in experimental models have varied from being beneficial, to detrimental, or insignificant.\(^1,3-6\) Most of the studies with beneficial outcome have been carried out with G-CSF being used prophylactically,\(^1,5,6\) however, pre-treatment with G-CSF is not practical under clinical conditions and it is the combination of G-CSF and antibiotic initiated at the time of therapy that is clinically relevant.

Nosocomial pneumonia is associated with virulent Gram-negative bacilli such as *Pseudomonas aeruginosa*, and the increase in antimicrobial resistance has provided a strong argument in favour of combination therapy.\(^7\) Novel strategies involving adjunct treatment with cytokines in combination with antibiotics may have promise due to the dearth of new antibiotics for Gram-negative infections.

The purpose of this study was to evaluate the efficacy of adjunctive G-CSF in the treatment of *P. aeruginosa* pneumonia when administered in combination with ceftazidime in both neutropenic (NT) and non-neutropenic (NN) hosts after the onset of infection.

Materials and methods

Microorganisms and drugs

A NCCLS quality control isolate of *P. aeruginosa* (ATCC 27853) with a ceftazidime MIC of 2 mg/L was used. The isolate was sub-cultured twice onto trypticase soy agar with 5% sheep blood (Becton Dickinson) at 37°C.
Efficacy of G-CSF in experimental pneumonia

before use in all experiments. Bacterial suspension was prepared from the second sub-culture that had been incubated for 18–24 h and was adjusted to >4.0 McFarland turbidity in a 5% dextrose saline solution, approximating 10^6 cfu/mL from which necessary dilutions were made for inoculation of the mice.

Ceftazidime (1 g vials (GlaxoSmithKline, Research Triangle Park, NC, USA) was reconstituted in normal saline and further dilutions made to obtain the required dosages. Recombinant human G-CSF (rhG-CSF) (Neupogen; Amgen Biologics, Thousand Oaks, CA, USA) was stored at 4°C until use. It was diluted daily as required in 5% dextrose. Dosages of all drugs were administered in 0.2 mL volumes.

Induction of lung infection

Female Swiss Webster mice (weight, 20–25 g; age, 30–60 days) obtained from Harlan Sprague Dawley (Indianapolis, IN, USA) were used for the study after approval from the hospital’s Institutional Animal Care and Use Committee. A group of mice were rendered neutropenic by intraperitoneal administration of cyclophosphamide 150 mg/kg on days -4 and -1 before bacterial inoculation whereas the NN group did not receive any cyclophosphamide. A suspension of ATCC 27853 was prepared as described above. Isofluorane (2%, v/v) in 100% oxygen carrier was used to induce a semi-anaesthetized state. Intratracheal inoculation was implemented to induce pneumonia by instilling a 0.05 mL bacterial suspension into the mouth and completely blocking the nares of the animal, thus resulting in bacterial inhalation through the mouth to the lungs. The mice were placed in an oxygen-enriched chamber for full recovery and thereafter randomized into various treatment groups.

Determination of minimum lethal dose (MLD) and antibiotic protective dose

The MLD (LD100) was determined by injecting groups of 10 mice (NT and NN) with serial dilutions of the bacterial suspension. Animals were observed and the lowest inoculum that produced 100% mortality within 3 days after infection was taken as the MLD.

To determine the dose of ceftazidime that results in not more than 50% protection against death after infection, NT and NN mice (10 per dose) were inoculated with 0.05 mL of ~5 × 10^7 cfu/mL and ~5 × 10^7 cfu/mL of the organism, which are their respective MLDs. Two hours after infection, the animals were given ceftazidime subcutaneously at a dose range of 125–2000 mg/kg followed by a second dose 4 h afterwards. Survival was recorded at four time points daily for 5 days.

Preliminary studies in this model involving bacterial density and mortality profile showed consistent growth by 1 h post-inoculation and proved the cause of death.

Therapy with G-CSF and antibiotic

The therapeutic effect of G-CSF was evaluated by inoculating NT and NN mice with the MLD of the organism and randomizing them into treatment groups (10 mice/group) that received 0, 50, 150, 300 and 400 µg/kg per day subcutaneous doses of G-CSF daily for 3 days, starting 2 h after infection. Control mice received only 5% dextrose. Survival was monitored daily for 5 days to evaluate the effect of combination therapy; both NT and NN mice infected with the MLD of the organism were randomized into treatment groups (15 per group) that received subcutaneous ceftazidime alone (2000 mg/kg × 2 doses); G-CSF alone (300 µg/kg per day for 3 days), or G-CSF + ceftazidime. The control group received normal saline and 5% dextrose. Animals were monitored daily for survival over 5 days. An additional study was carried out with 150 µg/kg per day of G-CSF in combination with ceftazidime in NN mice.

All determinations were carried out in duplicate or triplicate and pooled data were used for statistical analysis.

Statistical analysis

The minimum number of animals required in each treatment group to provide sufficient statistical power was determined by sample size estimation based on log rank test for a fixed time and constant hazard ratio. Time to death and percentage survival in both NT and NN mice were analysed by Kaplan–Meier Product, which was used to estimate mortality and comparison among treatment groups using the log rank test. A P value of <0.05 was considered significant.

Results

Therapeutic effect of G-CSF

Treatment with the MLD of the organism resulted in a dose-dependent increase in survival from 0 to 300 µg/kg per day compared with control in both NT and NN mice. Survival diminished with the 400 µg/kg per day dose approximating 0% by 48 h post-infection. The effect of G-CSF was more pronounced within the first 48 h after infection than afterwards. By the end of 5 days, the effect of G-CSF was no longer pronounced. The optimal dose of G-CSF was chosen to be 300 µg/kg per day since it produced the greatest survival although in the NN group, there seemed to be little difference between 150 and 300 µg/kg per day.

Efficacy of G-CSF–antibiotic combination

The therapeutic effect of combined administration of G-CSF and ceftazidime against P. aeruginosa infection is shown in Figures 1 and 2 for NT and NN mice, respectively. The doses of ceftazidime and G-CSF that maximally improved survival were chosen based on the dose range studies with each agent.

In the NT group, from 3 days after infection, survival was significantly higher (P < 0.02) in animals receiving the combination of G-CSF and ceftazidime versus the other groups and this difference was maintained until the end of the 5 day study. At the end of 5 days,
survival with G-CSF + ceftazidime was 64% compared with control (17%), G-CSF alone (25%) and ceftazidime alone (32%) ($P < 0.017$).

In NN mice, by the end of the 5 day study period, survival in the G-CSF + ceftazidime group was markedly higher than control and G-CSF alone ($P = 0.0001$). Although survival in the combination group (20%) was higher than ceftazidime alone (9%), the difference was not statistically significant ($P = 0.2020$). Combining 150 $\mu$g/kg G-CSF with ceftazidime did not improve survival ($P = 0.6116$) between ceftazidime alone and the combination.

There was no statistical difference between G-CSF and control ($P > 0.16$) in both groups.

**Discussion**

The dose-dependent effect obtained with G-CSF from 50 to 300 $\mu$g/kg per day coincided exactly with the period in which the infected mice received doses of G-CSF. By the end of 5 days, there seemed to be no benefit with G-CSF. Similar dose-dependent activity of G-CSF has been reported in some neutropenic animal infections involving enterococci and *P. aeruginosa*. This effect could be attributable to the degree of neutropenia as well as the level of infectious burden. The LD_{100} of *P. aeruginosa* used could be overwhelming for the host. Previous findings show that G-CSF was more beneficial with lower bacterial inocula than with higher inocula, an indication that the infectious dose of an organism significantly determines G-CSF treatment outcome. Dosing time is also important. G-CSF treatment was initiated after the mice were fully infected unlike previous studies.

The poor survival observed with a G-CSF dose of 400 $\mu$g/kg in both NT and NN mice is not fully understood since pathology of the lungs was not carried out but, from previous reports, it could be due to the presence of excess cytokine in the host, which can lead to replication of bacteria in the organs, reduction in platelet count or lung tissue damage.

This study demonstrates the beneficial effect of G-CSF as an adjunct when administered in combination with ceftazidime in the treatment of already established *P. aeruginosa* pneumonia despite the large inoculum of bacteria present. A previous study in our laboratory with the mucoid *P. aeruginosa* pneumonia model showed an adjunctive effect of azithromycin when combined with ceftazidime. The mechanism of interaction of G-CSF with ceftazidime is unknown, but the adjunctive effect with G-CSF may in part be due to immunomodulatory properties and anti-inflammatory factors. G-CSF enhances pulmonary recruitment of neutrophils and alveolar macrophages, enhancing their phagocytic activities as well as augmenting pulmonary host defences.

The reason for the non-significant finding in the NN host is unknown; therefore more invasive investigations may be required. G-CSF has demonstrated paradoxical findings in non-neutropenic models where it impaired rather than enhanced bactericidal activity of neutrophils.

In conclusion, in the case of post-infectious treatment of *P. aeruginosa* pneumonia, a combination therapy of G-CSF with ceftazidime may be beneficial as adjunctive therapy for treatment of such severe infections. This finding suggests new treatment options for the management of *Pseudomonas*-induced pneumonia especially in neutropenic patients. Clinical studies will have to further support this.

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**References**


