Pharmacokinetics of Subcutaneous Recombinant Human Granulocyte Colony-Stimulating Factor in Children

By N. Stute, V.M. Santana, J.H. Rodman, M.J. Schell, J.N. Ihle, and W.E. Evans

Fifteen children (age 1.2 to 9.4 years) with advanced neuroblastoma were treated with myelosuppressive chemotherapy (cyclophosphamide, cisplatin, doxorubicin) followed by 5-fluorouracil once daily for 10 days, starting the day after chemotherapy. Serial serum samples obtained on days 1 and 10 were analyzed for G-CSF activity by a specific proliferation assay using NFS-60 cells. G-CSF serum concentration-time data were best described by a one-compartment model, with zero-order absorption and first-order elimination. After SC injection, absorption was prolonged, with peak concentrations of G-CSF (3 to 117 ng/mL) being reached after 4 to 12 hours. The relatively slow absorption, with a mean elimination half-life of 5.8 hours on day 1 and 4.5 hours on day 10, provided measurable G-CSF concentrations for the entire 24-hour dosing interval in all patients at each dosage level. The median apparent clearance of G-CSF on day 10 was significantly higher than on day 1 (0.57 ± 0.31 mL/min/kg, P = .02), and was positively correlated with the absolute neutrophil count (ANC) (r² = .33, P = .003). Systemic exposure to G-CSF was dose-related, but interpatient pharmacokinetic variability yielded overlap in area under the concentration-time curve (AUC) at all three dosage levels. Stepwise regression analysis showed that G-CSF AUC could be predicted by a model that includes g-CSF dosage and ANC on the day of administration (r² = .82, P = .0001).© 1992 by The American Society of Hematology.

With the use of intensive chemotherapy for disseminated neuroblastoma and other childhood cancers, myelosuppression has become a dose-limiting factor. Recombinant human granulocyte colony-stimulating factor (rG-CSF) is currently being evaluated for its ability to ameliorate myelosuppression and permit greater dose intensity for chemotherapy of neuroblastoma. A recent report in adults receiving rG-CSF after myelosuppressive chemotherapy for small-cell lung cancer has shown significant improvements in neutrophil response, rate of hospitalization, and infectious complications in patients treated with this cytokine. However, relatively little is known about the disposition of rG-CSF in humans and there are no published pharmacokinetic data in children. If the pharmacokinetics (eg, absorption and elimination) of rG-CSF vary widely among patients, different responses could occur in patients administering the same dosage. The objectives of our study were to characterize the disposition of subcutaneous (SC) rG-CSF in children with neuroblastoma and to assess the pharmacodynamics of absolute neutrophil response to rG-CSF therapy.

MATERIALS AND METHODS

Patients and treatment protocol. The patients on this protocol were newly diagnosed, previously untreated children, greater than 1 year of age, with advanced-stage neuroblastoma. All patients received aggressive chemotherapy on the St Jude Children's Research Hospital protocol NB8814, which comprised cyclophosphamide 150 mg/m² intravenously (IV) (days −9 to −3), cisplatin 90 mg/m² IV (day −2), and doxorubicin 35 mg/m² (day 0) during the first course of therapy. All patients were administered rG-CSF at a dosage of 5, 10, or 15 μg/kg SC on days 1 through 10 after chemotherapy. Five children each were sequentially enrolled at the three dosage levels. A complete blood count (CBC) and differential were obtained before start of chemotherapy, as were serum albumin, creatinine, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and bone marrow aspirate. CBC and differential were also obtained on days −9 to 11 (including days 1 and 10 of rG-CSF treatment, when CBCs were performed before the rG-CSF dose was administered). This study was approved by the institutional review committee. Patients or parents gave informed written consent.

Pharmacokinetic study design. Pharmacokinetic studies of G-CSF were conducted on days 1 and 10 of rG-CSF therapy. Blood samples were drawn before and 0.25, 0.5, 5, 1, 2, 4, 8, 12, and 24 hours after the SC injection of rG-CSF. Whole blood was kept at 4°C, centrifuged within 3 hours, and serum kept at −20°C until assayed. rG-CSF. The G-CSF product used was provided by Amgen (Thousand Oaks, CA). It is a recombinant human protein expressed in Escherichia coli in nonglycosylated form, with a molecular weight of 18,627 daltons and a specific activity of 2 × 10⁶ U/mg protein.

Materials and reagents. A purified goat polyclonal antibody against rG-CSF expressed in E. coli was obtained from R & D Systems (Minneapolis, MN). RPMI-1640 media and L-glutamine were obtained from Whittaker Bioproducts (Walkerville, MD). Fetal bovine serum (FBS) was obtained from HyClone Laboratories (Logan, UT). Murine interleukin-3 (mIL-3) was expressed in COS-1 cells. Drug-free human serum (DFHS) was obtained from BioRad Laboratories (ECS Division, Anaheim, CA). ³H-thymidine, with a specific activity of 2 Ci/mmol, was obtained from NEN Products (Du Pont, Boston, MA).

Bioassay of G-CSF. Serum samples were analyzed for G-CSF with a proliferation assay using NFS-60 cells, a murine leukemic cell line that is dependent on G-CSF or mIL-3 and proliferates

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in response to human G-CSF. The cell proliferation assay described by Shirafuji et al was modified as described below. The NFS-60 cells used in this study were a subclone selected based on its specific response to human G-CSF and lack of response to other human cytokines (granulocyte-macrophage CSF [GM-CSF], IL-1, IL-3, IL-6, erythropoietin [Epo], IGF-1, IGF-2 [insulin-like growth factors], macrophage CSF [M-CSF], leukemia-inhibitory factor [LIF]; Dr J. Ihle, unpublished data), thus providing a specific bioassay.

The RPMI-1640 culture medium contained 10% FBS, 1% or 2 mmol/L L-glutamine, and 33 U/mL IL-3. The assay medium was the same, but without IL-3 and with antibiotics (100 U/mL penicillin, 0.1 mg/mL streptomycin). Cells were passed twice a week and incubated at 37°C and 5% CO₂. Standards were made fresh every run from the G-CSF vials, which were kept at 4°C. Standards and negative controls were made in media without IL-3. Controls were run in each assay and were made with drug-free human serum.

NFS-60 cells (5 × 10⁴ cells/well, viability > 90%) and serial dilutions of standards and patient samples were incubated on microtiter plates for 1 day. The proliferation in response to G-CSF was measured by incorporation of tritiated thymidine. After incubation for 20 hours at 37°C, 5% CO₂, and greater than 95% humidity, the cells were pulsed with 1 μCi [³H]-thymidine and incubated for another 5 hours. The cells were then harvested and the incorporated radioactivity measured by scintillation counting. All samples were analyzed in duplicate.

A sigmoid model was fit to the disintegrations per minute (DPM) for incorporated [³H]-thymidine versus rG-CSF concentration for standards (ie, assay calibrators) using ALLFIT software from the National Institutes of Health (NIH) and a template of the standard curve was used to fit the proliferation data for each patient sample. The concentration of G-CSF in each patient sample was measured by comparison of the concentration producing 50% maximum response (EC50) with the EC50 of calibrators.

The assay is highly sensitive and can detect concentrations as low as 0.005 ng/mL (0.27 pmol/L). Ninety percent of the maximal proliferative response was seen with this cell line at 1.2 ± 1.4 ng/mL (65 pmol/L) rG-CSF. Intrassay and interassay coefficients of variation were 12% and 18%, respectively, at 3.9 and 24 ng/mL rG-CSF. Specific activity of rG-CSF in the assay used was 35 U/µg. Day 1 and day 10 data from each patient were analyzed separately, with the predose concentration standard curve was used to fit the proliferation data for each patient sample. The concentration of G-CSF in each patient sample was measured by comparison of the concentration producing 50% maximum response (EC50) with the EC50 of calibrators.

Pharmacokinetic data analysis. A one-compartment model, with zero-order absorption and first-order elimination, was fit to each patient's G-CSF concentration-time data, using a Bayesian algorithm with the ADAPT II software. The population priors were obtained from previously published G-CSF pharmacokinetic data from adults, but with the volume of distribution adjusted for differences in body size for pediatric patients. Data from days 1 and 10 were analyzed separately, with the predose concentration treated as an initial condition.

The model was formulated as a closed form solution with the parameters: absorption rate (kₐ), apparent volume of distribution (Vₐ), and elimination rate constant (kₐ). Elimination half-life (t₁/₂ = 0.693/kₚ), apparent systemic clearance (Clₚ = kₚ × Vₐ), and area under the concentration-time curve (AUC = dose/Clₚ) were calculated from standard equations, and the maximum concentration (Cₘₐₓ) was the highest measured value in each patient.

Statistical analysis. The differences in absolute neutrophil count (ANC), G-CSF concentrations, and pharmacokinetic parameters between day 1 and day 10 were analyzed by the Wilcoxon signed rank test for patients with data on both days (n = 9). Associations between dosage of rG-CSF and pharmacokinetic variables on both days 1 and 10, and between dosage and neutrophil response were examined using the one-sided χ² rank test for an order. Stepwise linear regression was used to produce a model for the pharmacokinetic parameters G-CSF AUC and Clₚₚ. P < .05 was required to enter and stay in the model; observations with Rsudent greater than 3.0 were deemed outliers. Variables evaluated in stepwise regression analyses were rG-CSF dosage, ANC on study date, and patient characteristics before therapy (age, gender, ANC, serum albumin, bilirubin, ALT, AST, LDH, percent tumor cells in the bone marrow aspirate, cellularity, and percent myeloid cells). Stepwise regression was also used to assess the influence of the following parameters on ANC response (ie, ANC nadir, day of ANC nadir, and ANC fold increase): rG-CSF dosage, ANC on day 1, patient characteristics (see above), and pharmacokinetic parameters on day 1 (AUC, Clₚₚ, Cₘₐₓ, Vₐ, and 24-hour concentration).

RESULTS

Pharmacokinetics. Twenty-four pharmacokinetic studies were completed in 15 patients (12 females, three males). Twelve had disseminated disease with bone marrow involvement (stage D) and three had nodal disease (stage C). The age ranged from 1.2 to 9.4 years (median, 2.6). Serum creatinine (0.4 to 0.8 mg/dL), serum albumin (2.8 to 4.5 g/dL), and total bilirubin (0.1 to 0.5 mg/dL) were in the normal range. Nine patients had pharmacokinetic studies completed on both days 1 and 10 of rG-CSF therapy, four patients were studied only on day 1, and two patients only on day 10.

As shown in Fig 1, the G-CSF serum concentrations began to increase shortly after the SC injection, with no lag-time being observed. Absorption occurred over several hours, with peak concentrations being reached after 4 to 12 hours. A one-compartment model with first-order elimination and zero-order absorption fit the concentration-time data better than a one-compartment model with first-order absorption, based on a comparative analysis of residuals.

The median pre-dose concentrations (ng/mL) were considerably higher on day 10 (1.7; range, 0.2 to 20.5; 12 of 12 were detectable) than endogenous levels on day 1 before the first dose (0.011; range, undetectable to 0.120; 6 of 15 were undetectable). G-CSF peak (Cₘₐₓ) and 24-hour serum concentrations are summarized for each dosage level in Table 1. Despite considerable interpatient variability in G-CSF disposition, peak concentrations were positively associated with dosage, on both day 1 (P < .01) and day 10 (P = .01, n = 15). Differences in G-CSF concentrations were measurable in all 24-hour postdose samples at all three dosage levels, but there was large interpatient variability (range, 0.1 to 23.9 ng/mL) and the median concentrations on day 10 were lower than on day 1 (P = .04). With increased dosage of rG-CSF, clearance and Vₐ were significantly lower (P = .02 and P = .02, respectively) and 24-hour levels significantly higher (P < .01) on day 1. Similar, but nonsignificant, trends were seen on day 10. This
difference in clearance is not explained by differences in ANC at the three dosage levels. There was no statistically significant relationship between the dosage of rG-CSF and t1/2 on either day (P = .45, day 1; P = .59, day 10). G-CSF AUC was positively associated with dosage on both days 1 (P = .004) and 10 (P = .02), although pharmacokinetic variability resulted in an overlap in systemic exposure (ie, AUC) among the three dosage levels studied, with day 10 values being relatively lower than day 1 values (Fig 2).

Pharmacokinetic parameters estimated for all 24 studies in 15 patients are summarized in Table 2. The median apparent clearance of G-CSF from serum on day 10 was significantly higher than on day 1 (0.57 v 0.31 mL/min/kg, P = .02), and, consequently, the dose-normalized AUC was lower on day 10 (547 v 294 ng/mL x h, P = .01), when the nine patients with paired data were compared (Fig 3). No significant differences between days 1 and 10 were noted for the t1/2 (P = .12) and the Vd (P = .07).

In a stepwise regression analysis, G-CSF Clapp was significantly associated with the patients' ANC on the day of study and the rG-CSF dosage, accounting for 50% of the variability seen in G-CSF clearance after SC injections. The correlation between the clearance of G-CSF and ANC alone was low but significant (r² = .33, P = .003, n = 24), with greater clearance associated with higher ANCs.

**Neutrophil response.** The effect of chemotherapy and rG-CSF on the ANC in course 1 is shown in Fig 4. The median ANC (×10⁹/L) before chemotherapy was 2.9 (range, 0.6 to 11.0) and decreased to 1.9 (range, 0.4 to 6.1) after 10 days of chemotherapy (before starting rG-CSF). On day 10 of rG-CSF therapy the median ANC recovered to 5.6 (range, 0 to 19.8). However, there was large interpatient variability on all days. The increase of day 10 versus day 1 ANC levels was significant (3.0-fold; range, 0.1 to 10.9; P = .006), with several-fold increases in ANC occurring at all three dosage levels. The difference in ANC increase between the three dosage levels was not significant (P = .65).

The median ANC at nadir was 0.6 × 10⁹/L (range, 0.0 to 2.0) and the median nadir occurred on day 5 of G-CSF therapy (range, 0 to 8). In a stepwise regression analysis, the day of nadir was associated with the patients' age (r² = .52, P = .01), with older children reaching their ANC nadir earlier. For ANC at nadir and ANC fold increase (day 10 versus day 1), no significant variable was identified in stepwise regression analyses.

Stepwise regression analysis showed that G-CSF systemic exposure (ie, AUC) was significantly (P = .0001) associated with the rG-CSF dosage and the patients' ANC on the day of study, accounting for 82% of the variability seen in G-CSF AUC (Fig 5).

**DISCUSSION**

This study has shown several important characteristics of SC rG-CSF disposition in children with neuroblastoma. The pharmacokinetics of SC G-CSF were well described by a one-compartment, first-order elimination model with zero-order absorption. After the SC injection, absorption was prolonged, with peak concentrations of G-CSF occurring between 4 and 12 hours after injection. The relatively slow absorption and a mean t1/2 of 5.8 hours on day 1 and 4.5 hours on day 10 provided measurable G-CSF concentrations throughout the 24-hour dosing interval at each dosage level. These results support the administration of rG-CSF as a single daily SC dose in most patients. Despite considerable interpatient variability, there was a dose-related increase in the G-CSF AUC, consistent with first-order elimination (ie, linear pharmacokinetics) over the 5 to 15 μg/kg dose range. Within patients, G-CSF clearance increased significantly from day 1 to day 10 of G-CSF therapy, during which time there was a significant increase in ANC.

A stepwise regression model that includes the ANC on the day of study and the dosage of rG-CSF explained 82% of the variability seen in G-CSF AUC (P = .0001). This model builds on data from the present study and others,7-11 in which a lower AUC of G-CSF was observed in individual patients after an increase in ANC or a reduction in dosage.

Half the variability seen in G-CSF clearance was explained by a stepwise regression model that includes the ANC on the day of study and the dosage of rG-CSF (r² = .50, P = .001). The higher apparent clearance of G-CSF at lower dosages could be related to less complete SC absorption at lower dosages (due to local clearance at
Fig 2. Relationship between rG-CSF dosage and systemic exposure (AUC) after SC rG-CSF at three different dosage levels, both on day 1 (●), P = .004 (n = 13), and day 10 (△), P = .02 (n = 11).

the injection site) or less efficient systemic clearance at higher dosages (due to saturation of an elimination process). Either reduced absorption or enhanced removal from serum would increase the apparent clearance after SC injection of G-CSF. Because the t1/2 was not different at the three dosage levels studied (Table 1), it is likely that the higher Clapp at lower dosages is related to differences in absorption and not elimination. However, this finding requires further study.

No direct relationship was found between the dosage or systemic exposure (AUC) to G-CSF and the neutrophil response (ANC fold increase, ANC at nadir, duration of neutropenia). However, this is not unexpected, because ANC response is influenced by factors other than G-CSF exposure, such as the patient's tolerance to chemotherapy and the proliferative status of each patient's bone marrow.

The pharmacokinetic parameters reported for the pediatric patients in the present study are similar to previously published G-CSF pharmacokinetic data in adults.7-11 Peak concentrations after an SC injection of G-CSF were achieved within 4 to 8 hours in adults,7 similar to the 4 to 12 hours observed in our patients. The t1/2 of G-CSF reported in adults was 1.3 to 7.2 hours after a 30-minute IV infusion of 1 to 60 μg/kg,7,9 and 3.4 to 5.0 hours for SC injections of 1 to 5 μg/kg.10 Layton et al8 found a prolonged t1/2 at higher doses (3.7 ± 1.1 hours at 10 to 60 μg/kg v 1.4 ± 0.3 hours at 1 to 3 μg/kg), suggesting that a clearance mechanism becomes saturated at doses greater than 10 μg/kg. Our estimates of G-CSF half-life of 5.8 hours (day 1) and 4.5

Table 2. Summary of Pharmacokinetic Parameters for SC rG-CSF (5 to 15 μg/kg/d)

<table>
<thead>
<tr>
<th>Dosage SC (μg/kg)</th>
<th>Day 1 (n = 13)</th>
<th>Day 10 (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>0.31 ± 0.13</td>
<td>0.71 ± 0.66</td>
</tr>
<tr>
<td>AUC* (ng/mL x h)</td>
<td>624 ± 241</td>
<td>384 ± 231</td>
</tr>
<tr>
<td>Vapp (L/kg)</td>
<td>0.15 ± 0.08</td>
<td>0.24 ± 0.18</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>5.8 ± 2.1</td>
<td>4.5 ± 2.1</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD.
Abbreviation: n, number of patients studied.
*AUC normalized to a 10 μg/kg dosage.

Table 1. Median G-CSF Concentrations, Half-Life, Clearence, and Distribution Volume for SC rG-CSF (5 to 15 pg/kg/d)

<table>
<thead>
<tr>
<th>dosage SC</th>
<th>Cmax (ng/mL)</th>
<th>Cmax (ng/mL)</th>
<th>t1/2 (h)</th>
<th>VePp (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (n = 5)</td>
<td>15 (13-19)</td>
<td>16 (13-19)</td>
<td>2.1 (3.1-3.7)</td>
<td>0.15 (0.08-0.2)</td>
</tr>
<tr>
<td>10 (n = 5)</td>
<td>2.0 (1.0-2.5)</td>
<td>4.1 (2.5-8.1)</td>
<td>4.5 (5.0-6.7)</td>
<td>0.25 (0.06-0.3)</td>
</tr>
<tr>
<td>15 (n = 5)</td>
<td>63 (26-88)</td>
<td>78 (26-177)</td>
<td>5.0 (6.1-7.2)</td>
<td>0.30 (0.06-0.3)</td>
</tr>
</tbody>
</table>

Numbers in parenthesis are the range of values.
Abbreviation: n, number of patients studied at each dosage level; all other abbreviations defined in Materials and Methods.

Fig 2. Relationship between rG-CSF dosage and systemic exposure (AUC) after SC rG-CSF at three different dosage levels, both on day 1 (●), P = .004 (n = 13), and day 10 (△), P = .02 (n = 11).
Fig 3. Medians (solid lines in box), 25th and 75th percentiles (bottom and top of box), and range (upper and lower bars) for rG-CSF clearance and AUC on day 1 (■) and day 10 (△). Clearance is the apparent systemic clearance (Cl\text{sys}) assuming 100% SC absorption and AUC is normalized to a dosage of 10 μg/kg. Data are presented for nine patients with complete pharmacokinetic studies on both day 1 and day 10. P value for Wilcoxon signed rank test.

Fig 4. ANC measured in all patients during cytotoxic chemotherapy (days −9 to 0) and during rG-CSF therapy (days 1 to 10). Chemotherapy as described in Materials and Methods. rG-CSF treatment consisted of either 5, 10, or 15 μg/kg SC. Shown are means ± SD for the three dosage levels taken together. Missing values have been imputed using linear interpolation. The median number of ANC measurements in patients was 10; one patient was excluded due to sparse data.

Fig 5. Relation between G-CSF AUC measured and predicted by the stepwise regression model: AUC = \(-142 + 86.7 \times \text{dosage} - 30.9 \times \text{ANC}\); \(r^2 = .82, P = .0001\), n = 23. Higher G-CSF AUC (ng/mL \times h) was associated with a higher rG-CSF dosage (μg/kg) and lower ANC (\(\times 10^9/L\)), (●) Day 1, (△) day 10 of rG-CSF. One outlier was excluded from analysis.

In summary, the present study of rG-CSF in children has demonstrated favorable pharmacokinetic characteristics after SC injections. Absorption began immediately after administration and peak concentrations were reached 4 to 12 hours after the injection, consistent with a zero-order absorption process. Clearance was correlated with ANC, with greater clearance associated with higher ANC. Despite considerable interpatient variability in clearance, measurable G-CSF serum concentrations of greater than 0.1 ng/mL were produced for the entire 24-hour dosing interval in every patient at all three dosage levels.

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