Dose Escalation Study of Recombinant Human Granulocyte-Colony-stimulating Factor (KRN8601) in Patients with Advanced Malignancy

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ABSTRACT

To evaluate the toxicity and efficacy of recombinant human granulocyte-colony-stimulating factor (rh G-CSF) administered with intensive chemotherapy, 39 patients with advanced pulmonary cancers were enrolled in a dose escalation trial of rh G-CSF. Three days after initiation of chemotherapy rh G-CSF was administered i.v. for 14 consecutive days at five dose levels (50–800 µg/m²).

Absolute neutrophil counts showed a dose-dependent increase with an increasing dose of rh G-CSF and the durations of neutropenia (less than 1000/µm³) shortened significantly at doses of 200, 400, and 800 µg/m² compared to those at 50 µg/m² (P < 0.01). The duration of neutropenia was shortened significantly at all five dose levels following treatment with rh G-CSF compared to treatment without rh G-CSF (P < 0.05).

Adverse side effects associated with rh G-CSF administration were fever higher than 38°C (21%), chest pain, and low back pain (13%). No intolerable side effects were experienced. It can be concluded that rh G-CSF is effective in shortening the duration of neutropenia following intensive chemotherapy at a dose level of 100 to 200 µg/m² i.v. and a 400-µg/m² dose of rh G-CSF is recommended in patients with prior treatment because of the possibility of a lower bone marrow response.

INTRODUCTION

Intensive chemotherapy for patients with advanced lung cancer still presents a number of challenges. Bone marrow toxicity is one of the most hazardous side effects of intensive chemotherapy. Recently recombinant forms of four human hematopoietic colony-stimulating factors have been purified (1–3). The potential clinical applications of human hematopoietic colony-stimulating factors in cancer chemotherapy seem to be very promising. Reports on the effect of rh G-CSF and recombinant human granulocyte-macrophage-colony-stimulating factor in chemotherapy-induced neutropenia in patients with solid tumors have been published (4–9). However, the use of recombinant human colony-stimulating factors with intensive chemotherapy is still in the experimental stage. In this study we evaluated the efficacy and toxicity of rh G-CSF (KRN8601, Kirin:Amgen) (1) administered i.v. for 14 consecutive days at five escalating dose levels. The pharmacokinetics and bioavailability of rh G-CSF were also examined. The protective effect of rh G-CSF against bone marrow suspension was also analyzed in this study within the confines of a dose escalation study.

MATERIALS AND METHODS

rh G-CSF. rh G-CSF (KRN8601, Kirin:Amgen) was kindly provided by Kirin and Sankyo Company (Tokyo, Japan) (4). rh G-CSF

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1 Supported partly by Grant for Aid of the Comprehensive 10 Year Strategy for Cancer Control Japan.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: rh G-CSF, recombinant human granulocyte-colony-stimulating factor; NNR, neutrophil nadir ratio.

(KRN8601) has 174 amino acids and is in the nonglycosylated form with a molecular weight of 18,800. It was purified from Escherichia coli and the specific activity is 1.0 x 10⁶ units/mg of protein. There is no detectable endotoxin content.

Patient Selection. From January 1988 through September 1988 39 patients with advanced pulmonary tumors were enrolled in this study. The eligibility criteria were histologically or cytologically proved lung cancer or metastatic pulmonary tumor, Eastern Cooperative Oncology Group performance status of 0–3, normal liver and renal function, age between 15 and 80 years, and normal hematological function (WBC ≥ 4 x 10⁹/mm³, hemoglobin ≥ 10 g/dl, platelets ≥ 10 x 10⁹/mm³).

Patients were required to sign an informed consent indicating that they were aware of the investigational nature of this study in keeping with the policies of this hospital.

The ethics committee of the National Cancer Center Hospital gave their permission to conduct this protocol study.

Clinical Monitoring. The parameters evaluated were assessment of vital signs every 3 h, complete blood count and differential counts (three times per week during neutropenia), urinalysis, and tests for liver and renal functions twice a week. Chest radiographs were taken once a week to evaluate the response to chemotherapy.

In 10 patients the pharmacokinetics of rh G-CSF were evaluated using a double antibody radioimmunoassay. Briefly, plasma G-CSF was extracted by one step deproteinization with the use of isopropyl alcohol followed by the absorption to Bio-Rex 70 (Wako Pure Chemicals Industries Co., Ltd., Japan). The minimal detectable level of G-CSF in plasma was 0.25 ng/ml. The coefficients of variation of the intra- and interassays were 11.0 and 12.2%, respectively.

The formation of neutralizing antibodies to rh G-CSF was studied by obtaining blood samples before and after administration of rh G-CSF. Radioimmunoassay was used to detect the neutralizing antibodies.

Study Design. The safety and efficacy of rh G-CSF were evaluated in three to seven patients at each of five dosages. If WHO grade III–IV toxicity was observed at a given dosage, enrollment of an additional three patients at the same dose level was scheduled to evaluate the severity of the side effects.

Three days following intensive chemotherapy patients were given rh G-CSF in 100 ml of 5% dextrose solution i.v. over 30 min for 14 consecutive days. The initial starting dose of rh G-CSF in this study was 50 µg/m² and after evaluating the toxicity and change of neutrophil counts the doses of G-CSF were escalated to 100, 200, 400, and 800 µg/m². Seven patients received the same dose of rh G-CSF repeatedly. However, none of the individual patients received higher escalated dose.

In order to evaluate the effect of rh G-CSF combined with intensive chemotherapy, patients without prior chemotherapy were scheduled to receive a first cycle of chemotherapy without rh G-CSF and the second cycle of the same regimen with rh G-CSF. Then 2–3 cycles of the same chemotherapy with rh G-CSF were repeated if patients achieved partial response. Patients with prior chemotherapy were scheduled to receive a first cycle of chemotherapy with rh G-CSF and the second cycle of chemotherapy without rh G-CSF. For responders the same regimens with rh G-CSF were administered until disease progression (Fig. 1).

No other agents were mixed in the i.v. solution concurrently. The concurrent use of steroids or other immunomodulating drugs which

might influence the production of leukocytes in bone marrow was prohibited.

The chemotherapy regimens used in this study were: mitomycin C (8 mg/m²) plus vindesine (3 mg/m²) plus cisplatin (80 mg/m²), given to 21 patients with non-small cell carcinoma and 3 patients with metastatic pulmonary tumor; vindesine (3 mg/m²) plus cisplatin (80 mg/m²) and carboplatin (300 mg/m²) plus etoposide (120 mg/m² in 3 doses) given to 2 patients with non-small cell carcinoma; carboplatin (325 mg/m²) plus etoposide (160 mg/m² in 3 doses) given to 10 patients with small cell carcinoma; and cyclophosphamide (1 g/m²) plus Adriamycin (40 mg/m²) plus vincristine (2 mg/body) given to one patient with small cell carcinoma.

The pharmacokinetic data for rh G-CSF were analyzed using the program NONLIN84.

A statistical analysis (Student’s t test) was done to examine the differences between each rh G-CSF dose level.

RESULTS

The characteristics of the patients enrolled in this study are shown in Table 1. Thirty patients were male and 9 were female. Thirty-one were Eastern Cooperative Oncology Group performance status 0–1, and eight were 2–3. Thirty-six patients had primary lung cancer; 11 of them were patients with small cell lung cancer. There were 3 patients with metastatic pulmonary tumors, 2 from head and neck tumors and 1 from uterine cancer. Eleven patients had no prior therapy and 28 received previous treatment. Twenty patients received prior combination chemotherapy and 8 patients received prior radiation therapy (four patients to chest, three patients to head and neck, one patient to pelvis), but the interval from the previous treatment was more than 1 month. According to chest x-ray findings 2 patients showed atelectatic changes without evidence of acute inflammation and the other patients had peripheral nodular lesions. Two patients received 3 courses of rh G-CSF and 5 patients received 2 courses of rh G-CSF.

Neutrophil Response and Duration of Neutropenia. Absolute nadir counts of neutrophils at each rh G-CSF dose level are shown in Table 2. Using the general linear models procedure with SAS regression analysis, there was a significant linear correlation between the dose levels of rh G-CSF and the absolute nadir count of neutrophils. NNR were calculated by dividing the neutrophil nadir count by the neutrophil count prior to chemotherapy administration. A high NNR demonstrates that leukocytopenia was less severe with rh G-CSF administration. At the dose level of 400 µg/m², the absolute nadir counts of neutrophils and the NNRs in patients with prior chemotherapy were significantly lower than those with no prior treatment [336 ± 350 (SD) versus 2659 ± 3086/mm³, 0.10 ± 0.09 versus 0.65 ± 0.73, respectively] and the duration of neutropenia [4.8 ± 3.0 versus 1.7 ± 2.7 days] was also significantly longer [P < 0.05, two-sided].

There were no significant differences in duration of bone marrow recovery with sequential cycles of rh G-CSF administration among seven patients who received more than two cycles of rh G-CSF.

Comparison of Chemotherapy-induced Neutropenia with or without rh G-CSF. There were 24 patients who received the same chemotherapy regimen both with and without rh G-CSF. The nadir count of leukocytes and the duration of neutropenia after chemotherapy with and without rh G-CSF were compared. Table 3 shows the NNR at each rh G-CSF dose level after the same chemotherapy difference in the mean NNR following chemotherapy with or without rh G-CSF at the levels of 50 and 800 µg/m² (P < 0.05, one-sided). No statistical differences were shown at the levels of 100, 200, and 400 µg/m².

As shown in Table 3, all dose levels showed a significant difference in the duration of neutropenia following chemotherapy administration with or without rh G-CSF. There were no differences in platelet and RBC counts during chemotherapy with or without rh G-CSF (data not shown).

Adverse Effects of rh G-CSF. There were eight patients with fever (≥38°C) which started 4–8 days after starting rh G-CSF and returned to normal when rh G-CSF cycles had finished (Table 4). We excluded febrile patients who had obvious evidence of acute infection such as positive bacterial culture. Three patients among these eight febrile patients received the same chemotherapy regimen both with and without rh G-CSF. They had no febrile episodes during chemotherapy without rh G-CSF. Four patients among these eight febrile patients had fever during leukocytosis with rh G-CSF administration and the other four patients showed fever during leukopenia with G-CSF period. There were no significant differences in the duration of febrile period or antibiotic administration between chemotherapy with and that without rh G-CSF. Five patients complained of chest pain and/or low back pain. Only three patients required analgesics. There was no obvious relationship between the dose of rh G-CSF and the frequency of these
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Table 3 NNR and duration of neutropenia in chemotherapy with or without rh G-CSF

<table>
<thead>
<tr>
<th>rh G-CSF (µg/m²)</th>
<th>NNR (mean ± SD)</th>
<th>Duration (mean days ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>G-CSF(+)</td>
</tr>
<tr>
<td>800</td>
<td>5</td>
<td>2.18 ± 1.55</td>
</tr>
<tr>
<td>400</td>
<td>6</td>
<td>0.61 ± 0.95</td>
</tr>
<tr>
<td>200</td>
<td>7</td>
<td>0.32 ± 0.46</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>0.31 ± 0.34</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>0.15 ± 0.12</td>
</tr>
</tbody>
</table>

a NNR/pretreatment neutrophil count.
* One-sided test.
' Two-sided test.

Table 4 Side effects associated with rh G-CSF

<table>
<thead>
<tr>
<th>Side effect</th>
<th>Number of patients (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (≥38°C)</td>
<td>8/39 (21)*</td>
</tr>
<tr>
<td>Chest pain, low back pain</td>
<td>5/39 (13)</td>
</tr>
<tr>
<td>Pain requiring analgesics</td>
<td>3/39 (8)</td>
</tr>
<tr>
<td>Skin rash</td>
<td>2/39 (5)</td>
</tr>
<tr>
<td>Electrocardiographic change (ST depression)</td>
<td>1/39 (3)</td>
</tr>
<tr>
<td>Transient elevation of serum lactate dehydrogenase (WHO grade III)</td>
<td>1/39 (3)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Table 5 Pharmacokinetics of rh G-CSF

<table>
<thead>
<tr>
<th>rh G-CSF (µg/m²)</th>
<th>Cmax (ng/ml)</th>
<th>γt (h⁻¹)</th>
<th>t½ (terminal) (h)</th>
<th>Area under curve (ng/h/ml)</th>
<th>Clearance (ml/min/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>55.0</td>
<td>0.050</td>
<td>13.9</td>
<td>391</td>
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<tr>
<td>200</td>
<td>76.0</td>
<td>0.132</td>
<td>5.3</td>
<td>444</td>
<td>7.5</td>
</tr>
<tr>
<td>400</td>
<td>82.0</td>
<td>0.172</td>
<td>4.0</td>
<td>427</td>
<td>7.8</td>
</tr>
<tr>
<td>800</td>
<td>204.0</td>
<td>0.050</td>
<td>13.9</td>
<td>1182</td>
<td>5.6</td>
</tr>
<tr>
<td>1888</td>
<td>108.0</td>
<td>0.113</td>
<td>6.2</td>
<td>902</td>
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<tr>
<td>544</td>
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<td>4.1</td>
<td>2289</td>
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<tr>
<td>800</td>
<td>500.0</td>
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<td>2215</td>
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</tr>
<tr>
<td>800</td>
<td>515.0</td>
<td>0.104</td>
<td>6.7</td>
<td>3709</td>
<td>3.6</td>
</tr>
<tr>
<td>800</td>
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<td>0.150</td>
<td>4.6</td>
<td>3189</td>
<td>4.2</td>
</tr>
</tbody>
</table>

rh G-CSF was administered by i.v. drip for 30 min. The pharmacokinetic study was done by radioimmunoassay. The pharmacokinetic data were analyzed using NONLIN84.

DISCUSSION

rh G-CSF offers the potential of minimizing the side effects of neutropenia following administration of intensive chemotherapy for lung cancer. There seem to be three major advantages for utilizing rh G-CSF: (a) to increase the absolute nadir count of neutrophils during chemotherapy; (b) to shorten the duration of neutropenia; and (c) to decrease the chance of infection as a hazardous complication of neutropenia. In the chemotherapy for lung cancer the current goal of rh G-CSF studies is to be able to give high dose chemotherapy or rapid sequence chemotherapy more safely. Especially in small cell lung cancer attempts to increase the cure rate with such strategies are of great interest.

The results of phase I/II studies of rh G-CSF combined with intensive chemotherapy have been published by three different institutions (4–6): Christie Hospital reports 12 patients with small cell lung cancer (4); Royal Melbourne Hospital reports 15 patients with advanced malignancy (15); Memorial Sloan-Kettering Cancer Center reports 27 patients with urothelial cancer (6). Those studies showed the neutrophil count increased in a dose-dependent manner and the duration of neutropenia significantly shortened with rh G-CSF. In this study we use the same rh G-CSF at dose levels similar to those of the three reports (50–800 µg/m² versus 1–40, 1–60, and 1–60 µg/kg). In our study, an 800-µg/m² dose of rh G-CSF was effective in increasing the absolute nadir count of neutrophils after chemotherapy administration. Three of these 6 patients showed nadir neutrophil counts higher than 1 x 10⁴/mm³, and at the 400-µg/m² level, 4 of 20 patients showed a maximum neutrophil count over 3 x 10⁴/mm³ during rh G-CSF administration. To compare the neutrophil counts of each patient prior to chemotherapy administration, we used NNRs for evaluating the bone marrow protecting effect of rh G-CSF. In our study the NNR was >1 at the dose level of 800 µg/m² of rh G-CSF. This implies

Fig. 2. Pharmacokinetic curve of rh G-CSF. rh G-CSF was administered by i.v. drip infusion for 30 min. The pharmacokinetic study was done with the radioimmunoassay.

Complaints. One patient showed abnormal ST segment depression in anterior precordial leads which disappeared after the discontinuation of rh G-CSF. This patient did not show leukocytosis during the episode of chest discomfort. Two patients developed a skin rash which spontaneously regressed within 5-7 days during the continuation of rh G-CSF treatment. In one patient the serum lactate dehydrogenase transiently increased (WHO grade III). In 9 patients the administration of rh G-CSF was stopped because of a high WBC (≥3 x 10⁹/mm³). Two of 6 patients at 800 µg/m² were included in this group. There was no evidence of antibody formation against rh G-CSF.

Pharmacokinetics of rh G-CSF. Pharmacokinetic curves of rh G-CSF are shown in Fig. 2. Parameters of pharmacokinetics using a two compartment model are shown in Table 5. The peak plasma concentrations of rh G-CSF at 100, 200, 400, and 800 µg/m² were 55, 79, 196, and 512 ng/ml, respectively, at 30 min after rh G-CSF infusion. The plasma terminal half-life of rh G-CSF was 7.2 ± 3.7 h. The rh G-CSF areas under the curve at each dose level increased in a dose-dependent manner.
that rh G-CSF is biologically more active than clinically necessary at this dose level.

In patients with prior chemoradiotherapy treatment, there was some indication that the duration of neutropenia was prolonged compared to those with no prior treatment. In one other report the patients with prior pelvic irradiation showed a lower neutrophil nadir after rh G-CSF (6). It may be necessary to escalate the dose level of rh G-CSF in patients with previous treatment.

When rh G-CSF was combined with chemotherapy, the duration of neutropenia (WHO grade III, IV) shortened significantly at all dose levels compared to those without rh G-CSF.

rh G-CSF has been shown to enhance the proliferation of human nonhematopoietic tumor cell lines in vitro using the human tumor clonogenic assay (11). We examined the effect of rh G-CSF on the growth of human lung cancer cell lines using the double layer clonogenic assay. No antitumor activity or colony-stimulating activity in the colony formation was observed with lung cancer cell lines including two small cell lines (11).

According to these data we conclude that the optimal dose of rh G-CSF in patients with intensive chemotherapy for lung cancer is 100 to 200 μg/m² with a 30-min i.v. drip infusion for 14 consecutive days. In patients with prior treatment the recommended dose of rh G-CSF is 400 μg/m² because of the possibility of a lower bone marrow response.

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