Pharmacokinetics of Recombinant Human Interleukin 3 Administered Subcutaneously and by Continuous Intravenous Infusion in Patients after Chemotherapy for Ovarian Cancer

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ABSTRACT

Twenty chemotherapy-naive patients with ovarian carcinoma received 1, 5, 10, or 15 µg/kg/day (five patients per dose step) of recombinant human interleukin 3 (rhIL-3) over 7 days after carboplatin/cyclophosphamide in Cycles 1 and 3. Patients received rhIL-3 by continuous i.v. infusion or once daily s.c. injection in Cycle 1 and the alternate route in Cycle 3. Plasma rhIL-3 samples were obtained once daily on Days 1 to 6 and serially over a 24-h period on Day 7 for pharmacokinetic assessment of s.c. and i.v. administered rhIL-3 in 16 and 17 patients, respectively. Concentrations were assayed by a time-resolved fluorescence sandwich immunoassay. Pharmacokinetic parameters were derived by noncompartmental methods. Mean steady-state concentrations during continuous i.v. infusion ranged from 117 pg/ml (1 µg/kg/day) to 2217 pg/ml (15 µg/kg/day) and were linearly related to dose (r = 0.87, P < 0.001). When dose normalized, the mean steady-state concentrations were comparable at all doses. The total-body clearance was approximately 4 to 5 ml/min/kg. Elimination half-life (t½) could be assessed for the 5- to 15-µg/kg/day dose levels and was 53, 41, and 26 min for the 5-, 10-, and 15-µg dose levels, respectively (not significant between dose levels). Following s.c. injection, the maximum rhIL-3 plasma concentration ranged from 206 pg/ml (1 µg/kg/day) to 6930 pg/ml (15 µg/kg/day). Both the maximum measured plasma concentration (r = 0.89, P < 0.0001) and the area under the plasma concentration/time curve (r = 0.93, P < 0.0001) were related to dose. Dose-normalized values were comparable over the entire dose range. Elimination t½ was 4.8 h at the 1-µg/kg level and roughly half this time for the 5- to 15-µg/kg/day dose levels. The systemic clearance of approximately 5 to 6 ml/min/kg was comparable at all dose levels. Based on trough levels of the 7-day s.c. course, no rhIL-3 accumulation occurred. Bioavailability of s.c. administered rhIL-3 was nearly 100%. No correlation between creatinine clearance and pharmacokinetic parameters of rhIL-3 could be demonstrated. Since there was also no difference in hematological efficacy between the two routes of rhIL-3 administration, we conclude that the s.c. route of administration appears to have no disadvantages over the i.v. route and may facilitate its clinical application.

INTRODUCTION

IL-3 is a hematopoietic growth factor that promotes the survival, proliferation, and differentiation of multipotential hematopoietic stem cells and of the committed progenitor cells of the megakaryocyte, granulocyte/macrophage, erythroid, eosinophil, basophil, and mast cell lineages in vitro (1-4). It also affects mature cells by increasing cytotoxic functions or inducing release of cytokines, but does not affect the function of mature neutrophils (5, 6). Recent clinical studies with rhIL-3, administered to patients with advanced malignancies, myelodysplastic syndromes, aplastic anemia, or bone marrow failure (7-11), demonstrated its multilineage stimulation of hematopoiesis in vivo. These effects were especially prominent in patients with normal bone marrow function (7). Recently we have demonstrated that rhIL-3 administered once daily s.c. or by continuous i.v. infusion reduced the duration of chemotherapy-induced neutropenia and thrombocytopenia in patients treated for advanced ovarian cancer (12). A similar effect of rhIL-3 has been demonstrated in patients with relapsed small cell lung cancer treated with chemotherapy (13).

To date, the pharmacokinetic parameters of rhIL-3 have been studied in only a limited number of patients (10, 11). In addition, these patients were not being treated with chemotherapy. Therefore, in addition to evaluating the hematological effects and toxicity profiles (12), we studied the pharmacokinetics of rhIL-3 administered at doses of 1 to 15 µg/kg/day once daily s.c. and by continuous i.v. infusion at different doses in patients with ovarian cancer receiving cytotoxic treatment.

MATERIALS AND METHODS

Chemotherapy-naive patients, between 18 and 70 yr of age, with Stage III-IV ovarian cancer according to the International Federation of Gynecologists and Obstetricians and eligible for treatment with chemotherapy were entered. Patients with severe heart, lung, liver (serum total bilirubin, ≧40 µmol/liter), or kidney impairment (creatinine clearance, ≤50 ml/min) were excluded from the study, as were patients with a WHO Grade 3-4 performance score.

Chemotherapy comprised six courses of carboplatin (300 mg/m²) and cyclophosphamide (750 mg/m²), both given i.v. on Day 1 every 4 wk on an outpatient basis. Carboplatin (Bristol-Myers Squibb, Troisdorf, Germany), dissolved in 250 ml of 5% dextrose, was infused over 30 min, and cyclophosphamide (ASTA Pharma A. G., Frankfurt, Germany), dissolved in 250 ml of 0.9% saline solution, over 15 min.

The patients were divided into four groups of five patients each. After the first, third, and fifth chemotherapy course, each group received 1, 5, 10, or 15 µg/kg/day of rhIL-3 for 7 days (Days 5 to 11), starting 4 days after chemotherapy. Escherichia coli-derived nonglycosylated rhIL-3 (2 to 10 × 10⁶ units/mg of protein) was provided by Sandoz (Basle, Switzerland) in vials of 150 µg (for i.v. administration) and 300 µg (for s.c. administration). For s.c. administration rhIL-3 was reconstituted with 1 ml of sterile water. If the volume exceeded 4 ml the dosage was divided over both upper legs. For the daily i.v. infusion polypropylene syringes were used for each of two 12-h infusions, containing half of the daily rhIL-3 dosage, 96 mg (2 mg/ml) of human serum albumin and 0.9% saline solution with a total volume of 48 ml.

The patients were randomized for either once daily s.c. or continuous i.v. rhIL-3 administration in Cycle 1. In Cycle 3 the alternative route of administration was used. During Cycle 5 all patients received s.c. rhIL-3. Complete blood cell counts were performed on Days 1 (before chemotherapy), 8, 13, 15, and 22. Liver function tests were performed on Days 1, 8, 15, and 22. Creatinine clearance at entry was calculated from the creatinine levels in 24-h urine and in serum. The patient group in this study was part of the group described in the Phase I/II study with rhIL-3 administered to patients on...
chemotherapy treatment for ovarian cancer (12). The study was approved by the Medical Ethical Committee of the University Hospital of Groningen. Informed consent was obtained from all patients.

**Pharmacokinetic Assessment of rhIL-3.** Pharmacokinetic assessment of rhIL-3 was performed in Cycles 1 and 3. Blood samples for pharmacokinetic analysis of s.c. administered rhIL-3 were drawn prior to the first (0 h), second (24 h), third (48 h), fourth (72 h), fifth (120 h), and seventh (144 h) injection, and following the seventh (last) injection at 2, 3, 4, 6, 10, 13, 24, and 48 h. Blood samples for pharmacokinetic analysis of rhIL-3 administered by continuous i.v. infusion were drawn prior to the start of infusion (0 h), and at 24, 48, 72, and 168 h during the infusion. Additional blood samples were taken 0.5, 1, 2, 4, 8, 14, 24, and 36 h after discontinuation of the rhIL-3 infusion. Venous blood samples were collected in 10-ml Vacutainer tubes containing sodium heparin (Becton Dickinson, Meylan Cedex, France) and immediately put on ice. Within 30 min after collection the samples were centrifuged at 4°C, and the collected plasma was frozen in polystyrene vials at -20°C.

The quantitative measurement of rhIL-3 plasma levels was carried out using a time-resolved fluorescence sandwich immunoassay. The assay was basically as described previously (14), but with a streptavidin-europium conjugate (15). At the appropriate assay dilution, commercially available streptavidin-europium (Wallac Oy, Finland) was found to give the same results and was used in the analysis of the samples. Neither of the two monoclonal antibodies used in the sandwich immunoassay showed any cross-reactivity with nine other lymphokines (16). Calibration and quality control samples, containing known amounts of rhIL-3 in normal pooled human plasma and kept stored in aliquots at -20°C, were analyzed with every batch of patient samples. Calibration curves were constructed, and the concentrations of rhIL-3 in both quality control and patient samples were interpolated from a logit/log linearization of the calibration samples. The dynamic range of the standard curve was from 40 to 20,000 pg/ml of plasma, although no samples were found to have concentrations in the upper range of the curve. The limit of quantification, based on the lowest quality control sample with a coefficient of variation of less than 20%, was 50 pg/ml of plasma (n = 82). Any systemic bias in measured concentrations was calculated from the results of several quality control samples ranging in concentration from 50 to 15,000 pg/ml of plasma. The percentage of bias (mean-expected concentration/expected concentration) × 100 was found to vary from -2% to +15%. However, the bias measured showed no overall positive or negative trend, with respect to concentration. Three cycles of freezing and thawing of quality control samples at four different concentrations had no deleterious effect on the concentrations of rhIL-3 measured when compared to the value obtained when the samples had been freshly prepared.

The pharmacokinetic characteristics of rhIL-3 were derived by standard noncompartmental methods. The Cmax and the time of its occurrence (tmax) were compiled directly from the concentration/time data after s.c. administration. The mean Cmax during continuous i.v. infusion was calculated from the concentration data obtained over the 7-day continuous infusion course. The AUC was calculated by the trapezoidal rule over one dosing interval for the s.c. concentration data obtained over the 7-day continuous infusion course. The calibration samples of the 17 patients receiving rhIL-3 by continuous infusion were obtained from 16 and 17 patients, respectively. The median age of the patients in the s.c. and i.v. group was 60 and 59 yr, respectively (range, 21–69). Evaluable for the s.c. data were four, three, five, and four patients at the 1-, 5-, 10-, and 15-μg/kg/day rhIL-3 dose levels, respectively. For the i.v. data the number of evaluable patients at the different dose steps was four (1 μg), five (5 μg), five (10 μg), and three (15 μg). The other patients were lost for pharmacokinetic evaluation because of premature discontinuation of rhIL-3 administration due to rhIL-3-related side effects, accidental s.c. infusion of rhIL-3, or tumor progression.

**Results.** Plasma samples for s.c. and i.v. rhIL-3 pharmacokinetic analysis were obtained from 16 and 17 patients, respectively. The median age of the patients in the s.c. and i.v. group was 60 and 59 yr, respectively (range, 21–69). Evaluable for the s.c. data were four, three, five, and four patients at the 1-, 5-, 10-, and 15-μg/kg/day rhIL-3 dose levels, respectively. For the i.v. data the number of evaluable patients at the different dose steps was four (1 μg), five (5 μg), five (10 μg), and three (15 μg). The other patients were lost for pharmacokinetic evaluation because of premature discontinuation of rhIL-3 administration due to rhIL-3-related side effects, accidental s.c. infusion of rhIL-3, or tumor progression.

**Continuous i.v. Infusion of rhIL-3.** Analysis of the plasma samples of the 17 patients receiving rhIL-3 by continuous infusion...
yielded 15 pharmacokinetic profiles during the infusion and 13 profiles after stopping the infusion. Plasma concentrations were below the limit of quantification for two patients at 1 μg/kg/day both during and following the end of infusion and for an additional two patients (one at 1 and 5 μg/kg/day each) after stopping the infusion.

The Cmax ranged from 117 pg/ml at the 1-μg/kg/day rhIL-3 dose step to 2217 pg/ml at 15 μg/kg/day (Table 2). The relationship of Cmax versus dose was adequately described by a linear equation (r = 0.87, P < 0.0001). The t½s.c. could be assessed for the 5- to 15-μg/kg/day rhIL-3 dose levels, and the median values were 53 min at 5 μg/kg/day, 41 min at 10 μg/kg/day, and 26 min at 15 μg/kg/day. Although there appeared to be a trend toward a longer t½s.c. compared to the t½i.v., this difference was not significant (Wilcoxon's rank sum test for paired observations). CLs.c./f, was approximately 4 to 5 ml/min/kg at all dose levels.

No relationship between creatinine clearance and CLs.c./f (r = 0.36), t½i.v. (r = -0.32), or dose-normalized AUCs.c./f (r = -0.53) was found. Also, t½i.v. and dose-normalized AUCs.c./f did not appear to be influenced by liver function disturbances. In addition, the two patients experiencing dose-limiting toxicity at the 15-μg/kg/day rhIL-3 dose step had similar renal and liver functions as the other patients.

In Fig. 2 the median plasma levels during the 24-h period after the last s.c. injection and after discontinuation of the continuous i.v. infusion in the five patients at 10 μg/kg/day of rhIL-3 are demonstrated.

Effect of Route of Administration of rhIL-3 on Hematological Efficacy. To compare hematological efficacy of s.c. and continuous i.v. administered rhIL-3, the neutrophil counts during s.c. and i.v. administered rhIL-3 in patients evaluable for both s.c. and i.v. pharmacokinetics (one patient at 1 μg, two at 5 μg, five at 10 μg, and three at 15 μg) were compared on Days 1, 8, 13, 15, and 22 (Fig. 3). This was also done for the platelet counts (Fig. 4). No differences in hematological efficacy between the routes of administration on any of the days that blood cell counts were performed were observed. In addition, the hematological efficacy of s.c. and continuous i.v. administered rhIL-3 was compared with two control cycles (Cycles 2 and 4) of the same group of patients. For the s.c. and i.v. route both the neutrophil and platelet recoveries were hastened compared to the control cycles.

DISCUSSION

Recently, the importance of the pharmacokinetic properties of recombinant human hematopoietic growth factors has been stressed (17, 18). Limited information, only in patients not treated with chemotherapy, on the pharmacokinetic profile of rhIL-3 administered s.c. or i.v. is available (10, 11). This paper is the first on the pharmacokinetic profile of rhIL-3 administered after chemotherapy. Following once daily s.c. injection of rhIL-3 in the dose range of 1 to 15 μg/kg/day, no accumulation occurred during the 7-day course as assessed by predose concentrations. The difference in the t½s.c. between the 1-μg/kg/day dose step and the three higher dose levels may be due to the small sample numbers and outlier values. Likewise, the apparent deviation from dose proportionality for Cmax at 5 μg/kg/day was most likely due to outlier values given the fact that Cmax/dose was similar for dose steps below and above this level.

Our data indicate that the t½s.c. may decrease with increasing rhIL-3 dose. However, the values of this parameter must be treated as tentative, since retrospectively the sampling strategy was suboptimal for rigorously characterizing the elimination rate. In addition, the fact that, due to severe side effects, the i.v. infusion in two patients at the 15-μg/kg/day rhIL-3 dose step was discontinued prematurely may have affected the data at that dose step. Preliminary conclusions based on the more robust parameter CLs.c./f, which is derived from the entire dose-independent elimination over the dose range investigated here.
and the kidney, with the kidney appearing to be an active site of degradation of rIL-3. In 89% of patients with significant renal dysfunction, the clearance of continuous i.v.-infused recombinant human granulocyte-macrophage colony-stimulating factor was decreased during the last day of treatment compared to patients with a normal renal function (18). We were unable to relate rIL-3 pharmacokinetic parameters to the liver function or renal function of patients. However, no patients with very poor liver or renal function were entered in our study.

As reported earlier, rIL-3-related side effects were dose dependent (12, 13). Since C_{max}, AUC_{sc}, and C_{SC} increased linearly with rIL-3 dose, a relation between the severity of the side effects and the rIL-3 dose becomes more likely. Moreover, in our report on the hematological effects of rIL-3 administered after chemotherapy, there was a tendency toward a dose-response relationship between the administered rIL-3 dose and the hematological effects. However, the 15-μg/kg/day rIL-3 dose showed no superior effects over 10 μg/kg/day. In addition, the 15-μg/kg/day dose step demonstrated a more severe toxicity profile. With regard to the linear relationship between the rIL-3 dose and the AUC_{sc}, this suggests a biological optimum for s.c. administered rIL-3 at approximately 10 μg/kg/day.

Comparing the hematological data from patients evaluable for s.c. and continuous i.v. administered rIL-3 demonstrated similar efficacy for the two routes of administration. The results from our previous study, in which fifty cycles with rIL-3 were evaluated, also demonstrated no difference in efficacy (12).

In conclusion, s.c. administered rIL-3 demonstrated an excellent bioavailability, with a short half-life and equivalent hematological efficacy compared to continuous i.v. administration. Therefore, the s.c. route of administration appears to have no disadvantages over the i.v. route and may facilitate its clinical application.

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