Hematopoietic effects of recombinant human interleukin-3 and interleukin-6
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Effects of recombinant human interleukin-6 with or without recombinant human granulocyte-macrophage colony-stimulating factor before and after chemotherapy


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Abstract

**Purpose.** Bone marrow suppression, especially thrombocytopenia is a major impediment to chemotherapy dose-intensity. Both recombinant human interleukin-6 (rhIL-6) and to a lesser extent recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) have shown to stimulate thrombopoiesis. In this study the effects of a combination of rhIL-6 and rhGM-CSF were evaluated before and after chemotherapy.

**Methods.** Non-small cell lung cancer- and breast cancer patients were eligible. RhIL-6 was initially given in a dose of 2.5 ($n=3$) and escalated to 5.0 μg/kg/day ($n=4$), rhGM-CSF at a dose of 5.0 μg/kg/day in all patients. Two weeks before chemotherapy rhIL-6 + rhGM-CSF were started and administered for 1 week, then, 4 days after chemotherapy (mitoxantrone 10 mg/m² and thiotepa 40 mg/m²) rhIL-6 + rhGM-CSF were simultaneously given for 10 days. Chemotherapy was repeated every 3 weeks. These patients were compared with a historical group ($n=7$) treated with rhIL-6 as the only hematopoietic growth factor but otherwise identically.

**Results.** Flu-like symptoms, consisting of fever, headache and myalgia were reported frequently and appeared to be dose-limiting for the combination. Nausea and vomiting occurred in the pre-chemotherapy period and were associated with rhIL-6 administration. Before chemotherapy, both rhIL-6 alone and rhIL-6 + rhGM-CSF resulted in a similar increase in platelet numbers. RhIL-6 + rhGM-CSF lead to an increase in leukocytes, whereas rhIL-6 alone did not affect leukocyte numbers. After chemotherapy, the administration of rhIL-6 alone or rhIL-6 + rhGM-CSF did not result in differences with regard to the nadir or recovery of either leukocytes or platelets.

**Conclusions.** Stimulation of both platelets and leukocytes by rhIL-6 + rhGM-CSF before chemotherapy was evident. However, this combination did not show synergistic effects on hematopoiesis after chemotherapy. Compared to rhIL-6 alone, the tolerability of the combination was lower, due to a higher incidence of flu-like symptoms.
Introduction

With the development of recombinant hematopoietic growth factors, the amelioration of chemotherapy induced bone marrow depression has become one of the challenges of medical research. In regimens in which myelosuppression is dose limiting, the use of hematopoietic growth factors may result in increased chemotherapy dose intensity. Reduction in the depth and duration of neutropenia after standard chemotherapy has been achieved with recombinant human granulocyte colony-stimulating factor (rhG-CSF) [1-3] and with recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) [4-6]. Thrombocytopenia then often remains the dose-limiting factor. In several clinical studies recombinant human interleukin-3 (rhIL-3) [7,8] and rhIL-6 have demonstrated stimulatory effects on thrombopoiesis and to a lesser extent on leukopoiesis [9-11].

RhIL-6 is a pleiotropic cytokine with various functions. Its effects on hematopoiesis are broad, with stimulatory activity on early progenitors as well as on more differentiated cell types [12]. With respect to the latter, rhIL-6 has proliferative effects on megakaryocytes in vitro and in vivo with a subsequent increase in platelet numbers in preclinical experiments [13-24]. These results were confirmed in clinical non-randomized studies, which demonstrated a faster recovery of platelets for higher rhIL-6 doses after chemotherapy [11,25].

In vitro rhGM-CSF has stimulating activity on the proliferation of immature myeloid progenitor cells and on more differentiated myeloid cells. Apart from quantitative effects on leukocytes and platelets, rhGM-CSF administration also resulted in augmented functional activity of neutrophils and monocytes [26]. Reduction of chemotherapy induced myelosuppression by rhGM-CSF was demonstrated by several groups [4-6,27], and in a small randomized phase III study reduction of the severity of neutropenia and thrombocytopenia after combination chemotherapy was shown [28]. Synergism was suggested between rhIL-6 and rhGM-CSF as demonstrated in vitro with regard to enhancement of growth and differentiation of all myeloid lineages [29]. Animal data provided further evidence that combination, in this case, of rhIL-3 and rhGM-CSF resulted in increased...
neutrophil and platelet recovery after radiation induced bone marrow aplasia [30].

Based on these data, it was hypothesized that a combination of rhIL-6 and rhGM-CSF might also act synergistically with regard to chemotherapy induced thrombocytopenia and neutropenia. Until now, no data are available on the combined use of rhIL-6 and rhGM-CSF before and after chemotherapy in the clinical setting.

To define tolerability and to assess efficacy, these hematopoietic growth factors were administered in combination to patients with breast cancer and non-small cell lung cancer (NSCLC) before and after chemotherapy. RhIL-6 was administered in a dose known to be biologically active with limited toxicity. Based on a phase I study [10], the starting dose was 2.5 μg/kg/day and was escalated to 5.0 μg/kg/day. RhGM-CSF was used in a fixed dose of 5.0 μg/kg/day. The results obtained in this group were compared with a cohort of patients treated according to the same protocol, with rhIL-6 as the only hematopoietic growth factor [10].

**Methods**

**Patients.** Patients with stage III or IV breast cancer or disseminated NSCLC, between the ages of 18 and 70 years, who had received no more than one prior chemotherapy regimen, with a minimum life expectancy of more than 3 months, and with a Karnofsky score of 60 or more were eligible. At entry, a leukocyte count ≥3.0 × 10⁹/l and a platelet count ≥100 × 10⁹/l were required. Patients with severe heart, lung, liver (serum bilirubin ≥40 μmol/l) or renal impairment (serum creatinine ≥150 μmol/l) were excluded, as were patients with a history of serious allergic reactions, rheumatoid arthritis, generalized psoriasis, membranous glomerulonephritis or other autoimmune disease. Concomitant treatment with other hematopoietic growth factors, cytokines, salicylates or corticosteroids was not allowed. The study was approved by the Medical Ethical Committee of the University Hospital of Groningen. All patients gave their written informed consent for the study.

**Study design.** Escherichia coli derived rhIL-6 (10⁸ U/l protein) was provided by Novartis Pharma Ltd. (Basel, Switzerland) in 2 ml vials of 150 or 750 μg undissolved lyophilisate and
was reconstituted with 1 ml of sterile water. RhGM-CSF with a specific activity of $8 \times 10^6$ U/mg protein was provided by Novartis Pharma Ltd. (Basel, Switzerland). The vials containing 0.216 mg glycosylated rhGM-CSF, were reconstituted with 2 ml of the enclosed vehicle.

The study design is shown in Figure 1. Both growth factors were administered concurrently subcutaneously (sc) for 7 days, starting day 1. Fourteen days after the start of rhIL-6/rhGM-CSF chemotherapy was given intravenously as a bolus, consisting of mitoxantrone (Lederle, Belgium) 10 mg/m$^2$ and thiotepa (Lederle, Belgium) 40 mg/m$^2$ both dissolved in 100 ml 0.9% NaCl. After chemotherapy, rhIL-6 and rhGM-CSF were administered for 10 days in the same dose as pre-chemotherapy starting day 19. The starting dose of rhIL-6 was 2.5 mg/kg/day, with the intention to escalate to 5.0, 10.0 and 20.0 mg/kg/day depending on the occurrence of non-hematological toxicity. RhGM-CSF was given at a dose of 5 mg/kg/day and was discontinued in case the leukocyte number raised above 20 $\times$ 10$^9$/l. The planned number of patients at each dose level was at least three. The results of the combination were compared a cohort of patients (group B) who had received the same dose of rhIL-6, without rhGM-CSF, in an otherwise identical regimen [10,11].

Chemotherapy was scheduled every 3 weeks. In case of incomplete hematological recovery i.e. leukocytes <3 $\times$ 10$^9$/l and/or platelets <100 $\times$ 10$^9$/l chemotherapy was postponed with weekly intervals, for a maximum of 4 weeks, until bone marrow recovery. Prophylactic platelet transfusions were administered for levels below 20 $\times$ 10$^9$/l and red blood cell transfusion for symptomatic anaemia. The number of chemotherapy cycles depended on the tumour response, i.e. in case of progressive disease further treatment was discontinued. In patients with stable disease, partial response, or complete response a total of 6 chemotherapy cycles was foreseen.

The maximum tolerated dose of rhIL-6 and rhGM-CSF was defined as the doses preceding that at which at least two patients experienced non-hematological WHO grade 3-4 toxicity or debilitating toxicity leading to discontinuation of rhIL-6 and rhGM-CSF. Acetaminophen,
with a maximum of 3 g per day, was administered orally to prevent fever or flu-like symptoms. Anti-emetics (metoclopramide or ondansetron) were prescribed, if necessary, for nausea.

Blood pressure, pulse and temperature were measured twice weekly, as were haemoglobin concentration, platelet counts, leukocyte counts and differential counts. Once a week liver (ASAT, ALAT, alkaline phosphatase and gamma GT) and renal function tests (serum creatinine and BUN) were performed with subsequent determination of serum levels of sodium, potassium, LDH, calcium, total protein, albumin, glucose and total cholesterol. C-reactive protein (CRP, normal value <2 mg/l) and serum amyloid A (SAA, normal value <3 mg/l) were measured using enzyme-linked immunosorbent assays [31] and determined at days 1, 5 and 15 in the pre-chemotherapy period.

**Statistical Analysis.** The two-tailed Wilcoxon test was used for statistical analysis. The Chi-squared test was used to discern differences in discrete variables. P values <0.05 were considered significant. Unless otherwise stated the two-tailed Wilcoxon test was used.

**Results**

Patient characteristics. Three patients received a rhIL-6 dose of 2.5 mg/kg/day and four patients received 5.0 mg/kg/day, both in combination with rhGM-CSF (group A). Because of intolerable toxicity no further escalation of the rhIL-6 dose was established. The results of this group (n=7) were compared with a historical control group of seven patients who had received rhIL-6 only, comprising three patients at 2.5 μg/kg/d and four at 5.0 mg/kg/day (group B). As shown in Table 1, both groups were equally balanced with regard to age, disease and prior treatment.

A total of 11 chemotherapy cycles were administered in group A and 17 in group B. As no differences were observed between a rhIL-6 dose of 2.5 and 5.0 μg/kg/day with respect to hematological effects, these doses were taken together in the analysis.
Non-hematological toxicity. The major side-effects which occurred in the first chemotherapy course are listed in Table 2. For all patients these symptoms were equal or less severe in the pre-chemotherapy period compared to the post-chemotherapy period. No grade 3-4 toxicity was observed in the pre-chemotherapy period. Fever, headache and flu-like symptoms were reported by all patients. Nausea and vomiting occurred during rhIL-6 ± rhGM-CSF administration, before as well as after chemotherapy. Every patient experienced erythema at the injection site. Due to intolerable symptoms consisting of a combination of headache, flu-like symptoms and nausea and vomiting, two patients (group A) discontinued treatment after the first chemotherapy cycle. These patients received rhIL-6 as well as rhGM-CSF, both in a dose of 5.0 μg/kg/day.

No changes were observed in renal or hepatic function during the study, except for one patient in group B in whom worsening of liver function parameters was considered to be due to progressive metastatic disease.
Table 2. Patients experiencing non-hematological toxicity, after chemotherapy (according to WHO-criteria).

<table>
<thead>
<tr>
<th>Symptom</th>
<th>rhIL-6/rhGM-CSF</th>
<th>rhIL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>No. of patients</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grade 1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>grade 2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grade 1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>grade 2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>grade 3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Flu-like symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grade 1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>grade 2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>grade 3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grade 0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>grade 1-2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>grade 3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Erythema at injection site</td>
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<td></td>
</tr>
<tr>
<td></td>
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**Hematological effects.** The analysis of hematological parameters was performed in the pre-chemotherapy period and the first post-chemotherapy period. In Table 3 the incidence of hematological toxicity is listed.

Pre-chemotherapy, the maximum levels of leukocytes (mean±SEM) in group A were reached at day 5, 18.0±3.3 vs 6.6±0.6 ×10⁹/l for group B (p=0.047). The increase in leukocytes in the pre-chemotherapy period in group A was predominantly caused by increases in the number of neutrophils and lymphocytes. After chemotherapy the
Effects of rhIL-6 with or without rhGM-CSF before and after chemotherapy

The number of leukocytes decreased. For group A the nadir was reached at day 27 with a mean (±SEM) of $1.8±0.4 \times 10^9/l$ versus day 30 for group B with a mean (±SEM) of $1.9±0.3 \times 10^9/l$ (NS). The duration of the leukocyte recovery was approximately the same for both groups (Figure 2). No grade 4 neutropenia nor neutropenic fever was observed during this study.

The number of platelets demonstrated an increase from baseline to maximum levels on day 15, $423±23 \text{ and } 456±31 \times 10^9/l$ (mean±SEM) for group A and B respectively (both vs baseline $p=0.018$). This resulted however, not in a difference between the groups. After chemotherapy, the platelet nadir in group A was $57±14 \times 10^9/l$ (mean±SEM) and occurred on day 27, the nadir in group B was $115±35 \times 10^9/l$ and occurred on day 30. These differences were not significant (Figure 3).

During the whole study, grade 4 thrombocytopenia was observed in 7 out of 11 courses in group A versus 2 out of 17 courses in group B (NS). The number of platelet transfusions was 6 out of 11 courses in group A versus 1 out of 17 courses in group B ($p<0.025$, Chi-squared). No signs of bleeding were observed.

In the pre-chemotherapy period the mean (±SEM) hemoglobin concentration decreased from $125±3 \text{ g/l to } 115±3 \text{ g/l in group A (p=0.018)}$ and from $126±3 \text{ g/l to}$

<table>
<thead>
<tr>
<th></th>
<th>rhIL-6/rhGM-CSF</th>
<th>rhIL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Blood transfusions</td>
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<td>8</td>
</tr>
<tr>
<td>Grade 4 neutropenia</td>
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<td>0</td>
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<tr>
<td>Neutropenic fever</td>
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<td>0</td>
</tr>
<tr>
<td>Grade 4 thrombocytopenia</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Platelet transfusions</td>
<td>6</td>
<td>1*</td>
</tr>
</tbody>
</table>

* $p<0.025$
Leukocytes (x10⁹/l)

Figure 2. Leukocyte counts (mean±SEM) in patients who received rhIL-6 plus rhGM-CSF (●) and rhIL-6 alone (○), before and after the first chemotherapy course (CT). HGF: rhIL-6 ± rhGM-CSF.

* p<0.05 between the two groups.

Platelets (x10⁹/l)

Figure 3. Platelet counts (mean±SEM) in patients who received rhIL-6 plus rhGM-CSF (●) and rhIL-6 alone (○), before and after the first chemotherapy course (CT). HGF: rhIL-6 ± rhGM-CSF.

106±7 g/l in group B (p=0.028), without significant differences between both groups. After discontinuation of rhIL-6 with or without rhGM-CSF the haemoglobin concentration returned to near-normal levels. In the first chemotherapy course this reduction in hemoglobin concentration was more pronounced than before chemotherapy (Figure 4). The number of courses in which blood transfusions were administered for symptomatic anemia was 5/11 in group A and 8/17 in group B (NS).
Biochemical effects. Acute phase responses as determined by levels of CRP and SAA were determined on days 1, 5 and 15 of the pre-chemotherapy period. Baseline levels of CRP (mean±SEM) were the same for both groups, i.e., 13.7±5.7 (group A) vs 11.4±8.0 mg/l (group B). A CRP increase was observed for both groups at day 5, 288.6±30.6 (p<0.001) 260.0±31.6 mg/l (p<0.001) for group A and B respectively, without differences between the groups. The same pattern was observed for SAA. Baseline levels of SAA were 6.3±1.8 and 4.6±1.8 mg/l for group A and B respectively (NS). At day 5 these values increased to 292.9±96.0 (p=0.024) and 298.0±78.3 mg/l (p=0.021) compared to day 1, for group A and B respectively, without differences between the groups. Both CRP and SAA levels returned to baseline within 7 days (=day 15) after discontinuation of rhIL-6 with or without rhGM-CSF.

Discussion

This study describes the effects of combined administration of rhIL-6 and rhGM-CSF in humans. Dose-limiting toxicity was reached when rhIL-6 was administered in a dose of 5 μg/kg/day in combination with rhGM-CSF in a dose of 5 μg/kg/day, due to intolerable symptoms consisting of a combination of headache, flu-like symptoms and nausea and vomiting. The doses were lower than the maximum tolerated doses of rhIL-6 or rhGM-CSF as a single drug. In another report, higher combined doses of rhIL-6 and rhGM-CSF were given, but the same type of symptoms were reported [32].

Pre-chemotherapy, the addition of rhGM-CSF to rhIL-6 resulted in a sharp increase in the number of leukocytes, as could have been expected from rhGM-CSF alone. After chemotherapy no additional hematological effects from rhGM-CSF were
observed. Even the higher number of leukocytes at the start of the first chemotherapy course did not provide an advantage, as both groups had a similar leukocyte nadir and recovery rate after chemotherapy. It should, however, be mentioned that this chemotherapy regimen did not result in severe neutropenia. As no neutropenic fever was observed in either group, minor protective effects may have been obscured.

Before chemotherapy the number of platelets demonstrated an impressive increase, irrespective whether patients received rhIL-6 alone or in combination with rhGM-CSF. After chemotherapy the platelet nadir occurred earlier and appeared to be deeper for patients receiving the combination, but these differences were not significant. When all the courses were compared, more platelet transfusions were necessary for those receiving the combination (Table 3). So with regard to platelet numbers, the addition of rhGM-CSF to rhIL-6 was even detrimental compared to rhIL-6 alone. Similar results were obtained by O'Shaughnessy et al., for the combination of rhIL-3 (instead of rhIL-6) and rhGM-CSF after chemotherapy; lower platelet counts compared to controls were reported after the combination [33]. These data are, however, in contrast with data from Budd et al., in which rhIL-6 (0, 1.0, 2.5 or 10.0 μg/kg/day) and rhG-CSF (5.0 μg/kg/day) were administered for 10 days starting the day after chemotherapy (MAID). This combination resulted in less courses with grade IV thrombocytopenia and subsequently in a reduced number of platelet transfusions [34]. Whether this difference, compared to our data, is the result of the fact that rhG-CSF was used instead of rhGM-CSF, the administration schedule or the chemotherapy regimen remains unclear. In the non-human primate model the combined administration of rhIL-6 and rhGM-CSF or rhG-CSF did not yield additive hematological effects [24,35].

Administration of rhGM-CSF alone, before any chemotherapy, resulted in an initial decrease in number of platelets [27], and after chemotherapy rhGM-CSF may increase platelet nadir and affect platelet recovery [27,28]. In addition, Gianni et al. demonstrated only a trend for less platelet transfusions in patients treated with rhGM-CSF [36]. Administration of rhIL-6 after chemotherapy resulted in a faster platelet recovery, without affecting platelet nadir [11]. Therefore, our finding that combined administration of rhIL-6 and rhGM-CSF after chemotherapy resulted in reduced platelet numbers and subsequently more platelet transfusions compared to
rhIL-6 alone is rather unexpected. The fact that rhIL-6 and rhGM-CSF were started only 4 days after chemotherapy may be critical, better results might have been obtained if these factors had been administered directly after chemotherapy.

This study showed that the combination of rhIL-6 and rhGM-CSF after chemotherapy is associated with a high frequency of side-effects and does not result in superior hematopoietic recovery compared to rhIL-6 alone. Our aim to augment platelet recovery after chemotherapy with this combination remained unsuccessful. In a recently published paper, the combined administration of rhG-CSF and rhIL-3 seemed more efficacious. As this combination tended to augment multilineage engraftment after myeloablative chemotherapy [37]. Furthermore, the use of more specifically acting growth factors, such as the recently cloned thrombopoietin or megakaryocyte growth and development factor may prove to be a better stimulator of thrombopoiesis. Several groups have already demonstrated the effects of thrombopoietin after chemotherapy and radiotherapy [38,39] and recently its effect in humans was demonstrated before chemotherapy [40].

References


