Effects of Recombinant Human Interleukin-6 in Cancer Patients: A Phase I-II Study


To define the toxicity profile of recombinant human interleukin-6 (rhIL-6) and to study its effect on hematopoiesis, biochemical parameters, and other cytokines, rhIL-6 was administered in a phase I-II study to 20 patients with breast carcinoma or nonsmall cell lung cancer. RhIL-6 doses were 0.5, 1.0, 2.5, 5.0, 10, and 20 μg/kg/d, with at least three patients per dose level. RhIL-6 was administered 24 hours by continuous intravenous infusion followed by subcutaneous (SC) administration for 6 days, partly on an outpatient basis. RhIL-6-related side effects were fever, headache, myalgia, and local erythema. Starting at 2.5 μg/kg/d, these side effects were compounded by nausea, reversible increase in liver enzymes, and anemia. Flu-like symptoms were controllable up to and including 10 μg rhIL-6/kg/d with acetaminophen. RhIL-6 increased platelet counts with a decrease in mean platelet volume and increased leukocytes caused by neutrophil, monocyte, and lymphocyte increase, with an increase in T-cells and natural killer cells at 1.0 and 2.5 μg rhIL-6/kg/d. The reversible anemia was characterized by a decrease in serum iron, and an increase in ferritin and erythropoietin without reticulocytosis. RhIL-6 reduced total cholesterol levels and a dose-related increase of C-reactive protein and serum amyloid A plasma levels was observed. Serum IL-6 levels were increased, especially at 10 and 20 μg/kg/d, whereas no change in IL-1β and tumor necrosis factor α levels was observed. RhIL-6 can be administered with controllable side effects in this setting, up to and including a SC dose of 10 μg/kg/d on an outpatient basis, and has a promising stimulating effect on leukopoiesis and thrombopoiesis.

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at least two patients experienced World Health Organization (WHO) grade III or IV toxicity or life-debilitating toxicity leading to discontinuation of the rhIL-6 treatment. Blood pressure, pulse rate, and temperature were controlled frequently during the first 4 days; body weight was measured once daily. Acetaminophen, with a maximum of 3 g/d, was administered for fever (>38.5°C, measured axillary) and serum levels of sodium, potassium, calcium, total protein, albumin, glucose, total cholesterol, serum iron, and serum ferritin at dose level 20 μg/kg/d were determined on days 1, 3, 8, and 15. Serum erythropoietin levels were determined by radioimmunoassay (RIA; Sorin Biomedica, Stillwater, MN). BM aspiration was performed on day 1 and day 8 for BM culture; granulocyte-macrophage colony-forming units (CFU-GM) and erythroid burst-forming units (BFU-E) were cultured as previously described. In BM smears the myeloid-erythroid ratio (M:E ratio) was determined. Immunophenotyping of peripheral lymphocyte populations was performed on days 1 and 8. CD3, CD4, CD8, CD19, CD20, CD56, CD57, and CD25 positive cells were determined by fluorescence-activated cell sorting analyses (Becton Dickinson, Sunnyville, CA). Samples for fibrinogen, Ig levels (IgG, IgM, IgA) measured by Behring nephelometer (Behringwerke AG Diagnostica, Marburg, Germany), and antinuclear antibodies (ANA) measured by immunofluorescence technique were taken on days 1, 8, and 15. On days 1 through 5 and days 8, 10, 12, and 15, plasma samples were obtained before rhIL-6 administration, for C-reactive protein (CRP), serum amyloid A (SAA), and IL-6. IL-1β, and tumor necrosis factor α (TNFα). CRP (normal value, <2 mg/L) and SAA (normal value, <3 mg/L) levels were obtained using enzyme-linked immunosorbent assays (ELISA)32 and IL-6 (normal value, <10 ng/L) by using the B9 bioassay and ELISA33. TNFα (detection limit, 15 ng/L) was measured by enzyme-amplified sensitivity immunoassay (Medgenix Diagnostics SA, Fleurus, Belgium) and IL-1β (detection limit 10 ng/L) by ELISA (Cistron Biotechnology, Finebrook, NJ).

Statistical analysis. The two-tailed Student’s t-test and the Spearman rank analysis were used for statistical analyses. P values <.05 were considered significant. Unless otherwise stated the two-tailed Student’s t-test was used.

RESULTS

Patient characteristics. As shown in Table 1, 20 patients, with a mean age of 44.9 years (range, 24 to 63), were entered. Twelve received both chemotherapy and radiotherapy, 3 received only chemotherapy, and 5 received no previous treatment before rhIL-6 administration, for C-reactive protein (CRP), serum amyloid A (SAA), and IL-6. IL-1β, and tumor necrosis factor α (TNFα). CRP (normal value, <2 mg/L) and SAA (normal value, <3 mg/L) levels were obtained using enzyme-linked immunosorbent assays (ELISA)32 and IL-6 (normal value, <10 ng/L) by using the B9 bioassay and ELISA33. TNFα (detection limit, 15 ng/L) was measured by enzyme-amplified sensitivity immunoassay (Medgenix Diagnostics SA, Fleurus, Belgium) and IL-1β (detection limit 10 ng/L) by ELISA (Cistron Biotechnology, Finebrook, NJ).

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Table 2. Side Effects

<table>
<thead>
<tr>
<th>rhIL-6 Doses (µg/kg/d)</th>
<th>0.5</th>
<th>1</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV SC</td>
<td>IV SC</td>
<td>IV SC</td>
<td>IV SC</td>
<td>IV SC</td>
<td>IV SC</td>
<td>IV SC</td>
</tr>
<tr>
<td>No. patients</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
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</tr>
<tr>
<td>Fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO grade I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>WHO grade II</td>
<td>---</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Chill</td>
<td>---</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>---</td>
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<td>Headache</td>
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<td>2</td>
<td>1</td>
<td>2</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Myalgia</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Local erythema</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Local infiltrate</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Nausea</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Hepatotoxicity WHO grade I-II</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1</td>
<td>NA</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

± SE; day 1, 6.33 × 10^9/L ± 0.46; day 3, 9.38 ± 0.52, P < .001; day 5, 6.88 ± 0.55, P < .02; day 15, 7.12 ± 0.61, P < .05 (Fig 3). The increase in leukocytes initially consisted of an increase in neutrophils (day 3) and monocytes (days 3 and 8), whereas a significant increase in lymphocytes (dose 0.5 to 20 µg, n = 18, P < .05) occurred on day 15. The effect of rhIL-6 on lymphocytes was characterized, especially at the lower dose levels, by a maximum increase 7 days after cessation of rhIL-6. On day 8, immunophenotyping (Fig 4) showed an increase in T cells, natural killer (NK) cells, and cells expressing the IL-2 receptor at 1.0 to 2.5 µg/kg/d. This increase was not consistent at 5.0 to 10 µg/kg/d and there was a significant reduction of these cells compared with day 1 (CD4, CD8, CD25, P < .05; CD57, P < .02) at 20 µg/kg/d. There was no alteration in the levels of the immunoglobulins IgG and IgM, but there was a slight increase in IgA reaching a maximum level on day 15 (mean ± SE: 2.87 ± 0.53 g/L v 3.33 ± 0.83, P < .01). There was no effect observed for rhIL-6 on basophils or eosinophils.

Figure 5 shows the effect of rhIL-6 on hemoglobin (Hb). There was a dose-dependent rapid decrease in the Hb level that could not be explained by the amount of blood drawn from these patients. Serum Fe was not affected at the lowest rhIL-6 doses, but there was a rapid drop in serum Fe at higher doses. This drop in Hb and serum Fe was reversible after cessation of rhIL-6, without concomitant blood transfusion. Iron staining of the BM at the 10 µg/kg/d dose did not show any changes, and there was also no change observed in the percentage of sideroblasts (<3%). There was no reticulocytosis during or after cessation of rhIL-6 with maximum (for anemia-corrected**) values of reticulocytes of 0.5% to 1.3% at the 10 to 20 µg/kg/d dose levels. Serum lactic dehydrogenase (LDH) levels were unchanged. Erythropoietin levels showed an increase at all dose levels at day 3 versus day 1 (P < .05) and they increased until day 8 (P < .01). This effect was dose dependent (day 8, r = .56, P < .05, Spearman rank analysis). The mean percentage increase of erythropoietin at 0.5 µg/kg/d on day 8 compared with day 1 was

Table 3. Increase in Platelet Count per Dose Level rhIL-6

<table>
<thead>
<tr>
<th>Dose rhIL-6 (µg/kg/d)</th>
<th>Baseline Value ± SE (×10^9/L)</th>
<th>Maximum Value ± SE (×10^9/L)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>352 ± 35</td>
<td>444 ± 62</td>
<td>NS</td>
</tr>
<tr>
<td>1.0</td>
<td>266 ± 44</td>
<td>421 ± 59</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>2.5</td>
<td>284 ± 34</td>
<td>505 ± 69</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>5.0</td>
<td>238 ± 6</td>
<td>480 ± 43</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>10</td>
<td>273 ± 15</td>
<td>577 ± 86</td>
<td>NS</td>
</tr>
<tr>
<td>20</td>
<td>256 ± 32</td>
<td>743 ± 119</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

Fig 1. Effect of rhIL-6 on platelet counts. Each point represents the mean ± SE of pooled data at all dose levels. P values were < .001 (*) and < .002 (**).
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Fig 2. The maximum percentage platelet increase compared with baseline in all patients. Doses of rhIL-6 were 0.5, 1.0, 2.5, 5.0, 10, and 20 μg/kg/d. Each bar represents one patient. The response was dose related (r = .84, P < .002, Spearman rank analysis).

(mean ± SE) 47 ± 25 and at 20 μg/kg/d, 113 ± 65. There was a decrease of erythropoietin levels after cessation of rhIL-6 at all doses. Serum ferritin levels at the 20 μg/kg/d dose were increased 440% ± 284% at day 8, and decreased at day 15. The number of BFU-E and CFU-GM was not affected by 7 days rhIL-6 treatment. There was an observed increase of the M:E ratio (mean ± SE: day 1, 3.21 ± 0.45; day 8, 4.05 ± 0.59; P < .02).

Biochemical effects. Of 20 evaluative patients, 4 had a 2.7- to 6.1-fold increase in AST (serum glutamic oxaloacetic transaminase [SGOT]) and/or ALT (serum glutamic pyruvic transaminase [SGPT]). A 1.4- to 3.4-fold increase of alkaline phosphatase occurred in 5 and γGT increased 1.6- to 8.0-fold in 11 patients. In 5 of these patients, this was a solitary increase. The highest increase was observed at day 8; the increases were reversible after cessation of rhIL-6. Only 1 of the patients had liver metastases and of the 9 patients showing no changes in liver enzymes, 3 had tumor lesions in the liver. There was no relationship between the pretreatment

Fig 3. Effect of rhIL-6 on leukocytes (□), neutrophils (▲), and monocytes (●). Represented is the mean ± SE at 2.5- to 20-μg/kg/d dose levels. An asterisk indicates a significant difference versus baseline.

Fig 4. Effect of rhIL-6 on lymphocyte subsets. Represented are pooled results of the mean ± SE of the 1.0- and 2.5-μg/kg/d doses (n = 5). (□), Results at day 1; (■) results at day 8. An asterisk indicates a significant difference versus baseline: for CD3, P < .05; CD4, P < .01; CD8, P < .02; CD56, P < .02; CD57, P < .001.

Fig 5. Effect of rhIL-6 on Hb level. Values are expressed in percentage, compared with baseline level of 100%. Represented are mean values at 0.5 μg (□), 1.0 μg (▲), 2.5 μg (■), 5.0 μg (●), 10 μg (▲), and 20 μg (●). The response was dose related (r = .73, P < .002, Spearman rank analysis).
Fig 6. Effect of rhIL-6 on total cholesterol level. Represented is the mean ± SE at the doses 1.0 to 20 µg/kg/d. The asterisk indicates a significant difference versus baseline ($P < .001$).

Liver function and the increases in liver enzymes during rhIL-6 treatment. Serum albumin decreased at day 8 with $5.3 ± 0.7$ g/L (mean ± SE) at 10 µg/kg/d and $10.0 ± 1.0$ g/L at 20 µg/kg/d. Levels were normalizing at day 15. No effect was observed on serum creatinine. ANA increased in 1 patient at 0.5 µg/kg/d rhIL-6. This increase persisted in the months after cessation of rhIL-6. RhIL-6 markedly reduced total cholesterol (Fig 6). This effect is rapid, pronounced, dose related (day 1 v day 8; $r = .69$, $P < .01$, Spearman rank analysis), and reversible.

**Effects on acute phase proteins.** rhIL-6 induced a rapid dose-dependent increase in CRP levels (Fig 7). The CRP levels showed a slight decrease during rhIL-6 treatment. The CRP levels in all patients at the highest dose level (20 µg/kg/d) were lower than at 10 µg/kg/d rhIL-6. The same pattern was observed for the acute phase protein SAA (Fig 8). RhIL-6 also increased fibrinogen at all dose levels, with a 74% ± 14% increase on day 8 (mean ± SE; $n = 12$; $4.87 ± 0.46$ g/L v $8.28 ± 0.29$ g/L; $P < .001$).

**Effects on cytokines.** IL-6 was detected in plasma during IV administration starting at 1.0 µg/kg/d and incidentally during SC treatment (measured 24 hours after administration) at 5.0 µg/kg/d and at 10 and 20 µg/kg/d in levels up to 3,000 ng/L (Fig 9). There was no increase measured in IL-1β and TNFα in any patient.

**DISCUSSION**

This study shows that rhIL-6 is relatively well tolerated by cancer patients at doses up to and including 10 µg/kg/d with the following side effects: fever, chills, headache, myalgia, minimal local erythema at the injection site, nausea, reversible increase in liver enzymes, and anemia.

The fever and flu-like symptoms at doses of 0.5 to 10 µg/kg/d were manageable with acetaminophen and nausea was
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The maximum tolerated dose in this setting for outpatient treatment of cancer patients was considered to be 10 μg rhIL-6/kg/d. This was based on general malaise symptoms, fever, and anemia and not on the occurrence of WHO grade III or IV toxicity in two patients at the same dose level. These data are in accordance with the results of Weber et al.30 Based on the 11 patients treated, they considered 10 μg/kg/d to be the safely tolerated dose. Dose-limiting toxicity consisting of arrhythmia and hepatotoxicity was observed at 30 μg/kg/d. rhIL-6 treatment was cautioned against especially in patients with pretreatment liver dysfunction or extensive tumor infiltration.30 In the present study, reversible liver enzyme increase, unrelated to the presence of liver metastases or pretreatment liver dysfunction and no clinical evidence of arrhythmia, was observed in 11 of the 20 patients.

The rhIL-6 treatment resulted in a dose-related increase in platelets, reaching a maximum after cessation of rhIL-6. This increase was preceded by an initial decrease in platelets at day 3, as was also noticed by Weber et al.30 The origin of this initial decrease is still unknown. The increase in platelets coincided with a reduction in platelet volume.

rhIL-6 did increase leukocytes because of an increase in neutrophils and monocytes and this was not associated with an increased number of CFU-GM. It is conceivable that the increase may be related to the induction of other cytokines or to the cooperative effect of different cytokines because in vitro culture IL-6 can promote the differentiation of the monocytic pathway.42 The effect on lymphocyte subpopulations was dose dependent. An increase in T cells, NK cells, and cells expressing the IL-2 receptor was observed at the lower dose levels (1.0 and 2.5 μg/kg/d) and a reduction of the number of these cells at the highest dose level. Because of the limited number of patients at each dose step, it is difficult to conclude that these effects are clearly dose related.

An impressive effect on acute phase protein production was observed, which is in agreement with animal data26 and the recent reports in humans.36-38 Regarding the SAA, CRP, and fibrinogen response, it is interesting that SAA levels— and to a lesser extent CRP levels—already decreased during the rhIL-6 treatment after having reached a maximum at 48 hours. Although similar findings were reported by Mayer et al.29 in nonhuman primates with SC treatment only, this might be the result of the administration schedule with IV initially and then SC treatment. The initial acute phase response dur-
ing the first 48 hours was dominated by SAA, whereas the increase in SAA and CRP levels during later rhIL-6 treatment, especially at higher rhIL-6 doses, tended to be equal in all patients studied. This suggests that SAA may predomi- nate over CRP in acute situations, whereas CRP predomi- nates the response in chronic acute phase reactions. In conclusion, we have shown that rhIL-6 can be safely administered up to and including a dose of 10 \( \mu \text{g/kg/d SC} \) with side effects common to other biologic response modifiers. An important and unexplained side effect, anemia, warrants further study. The most important clinical effect of rhIL-6 in humans is thrombocytosis with a potentially beneficial effect on WBCs being observed in addition. Further studies into the efficacy of rhIL-6 alone and after chemotherapy should be performed.

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