Cytokine Kinetics: Clinical Pharmacology Studies Complementing Recombinant Growth Factor Trials

JOSH RABINOWITZ; WILLIAM P. PETROS, PHARMD; WILLIAM P. PETERS, MD, PHD

Hematopoietic regulation is the best known of the many functions of cytokines, a complex set of proteins that may also play a role in aiding immune response and healing wounds.1 Clinical trials have shown that recombinant hematopoietic growth factor (HGF) administration speeds hematopoietic recovery following myeloablative chemotherapy.2-15 Recombinant HGF may have the potential to modulate both its own endogenous expression and that of other cytokines. Exogenously administered and/or endogenously produced cytokines may also directly or indirectly contribute to chemotherapy-associated toxicities. Administration of proteins, such as recombinant tumor necrosis factor-alpha (TNF-α), produce some symptoms similar to side effects of chemotherapy.16,17

The net physiologic effect of circulating cytokines may depend on several factors, including cytokine concentrations and the presence of receptor antagonists and/or inhibitors (Figure 1). Soluble receptors may bind circulating protein messengers before they interact with cell-surface receptors, inhibiting the messenger’s effect. Receptor antagonists that block cell-surface receptors and compounds that down-modulate cell-surface receptors may also inhibit cytokine activity.

In response to the growing importance and complexity of recombinant HGF therapy, a veritable explosion in cytokine kinetic literature has occurred in the past few years. This article focuses on the highlights of some of the cytokine kinetic studies performed in our laboratory and their potential clinical implications.

Methods

Accurate determination of the in vivo activity of a recombinant HGF may require measurement of a variety of proteins. Cytokine concentrations can vary rapidly, both on daily cycles and as clinical conditions of the patient change. Consistent, frequent sampling is often necessary to account for these variations.

Sample handling, such as the time between sample collection and freezing and the number of freeze-thaw cycles to which a sample is exposed, may affect cytokine measurement. Uniform sample handling during the cryopreservation process is crucial to a well-controlled study. Dividing samples into aliquots, which will be used for multiple assays prior to storage at −70 C, prevents exposure of the sample to multiple freeze-thaw cycles.

Most current cytokine kinetic studies, including all studies in our laboratory, are performed by enzyme-linked immunosorbent assay (ELISA) techniques. (For many cytokines, ELISAs are commercially available.) Use of an automated plate reader allows the measurement of 96 samples in about 3 to 8 hours. Although ELISAs provide easy, quantitative measurement of cytokine concentrations, they measure the immunoactivity, not necessarily the biologic activity, of proteins. False-positive results are possible when a molecule rendered biologically inactive remains intact at the assay’s antibody binding site. However, biologic assays are often too cumbersome and variable for extensive studies of cytokines at multiple time points. Furthermore, they may be influenced by endogenous inhibitors sometimes found in clinical samples.

Patient selection is another important consideration in study design. Chemotherapeutic and supportive-care regimens may each have an impact on cytokine kinetics. Well-controlled comparisons between patients receiving near-identical treatments provide the optimal setting to determine cytokine interactions. Unlike some studies that have combined patients receiving different treatments, our studies consider only patients receiving the identical myeloablative regimen with autologous bone marrow support. The effect of differences in supportive care is explicitly addressed in each report.
Clearance Determinants

Elevated serum cytokine concentrations may result from exogenous drug administration, increased endogenous cytokine production, or reduced cytokine elimination. A variety of mechanisms appear to be important in cytokine elimination, including attachment of ligand to the cell-surface receptor and endocytosis; metabolism by proteolytic enzymes; and urinary excretion by glomerular filtration with subsequent reabsorption and catabolism (Figure 1). The pattern and pathways of elimination of exogenously administered cytokines may be affected by dose, administration route and schedule, degree of glycosylation of the recombinant protein, specific receptors available, and in some cases renal function. Presence of other proteins, such as receptor antagonists or modulators of receptor expression, may also significantly affect receptor-mediated clearance.

Cytokine production is thought to occur in a wide variety of hematopoietic cells and in other locations in the body. Studies indicate that concentrations of some cytokines increase during periods of neutropenia and have suggested that neutropenia causes their enhanced production. However, during periods of neutropenia, the number of receptors for cytokines may also be reduced, potentially altering cytokine elimination. Further work seeking direct evidence of increased cytokine production (e.g., increased production of messenger RNA for the cytokine in question) is required to clarify this important question.

Studies in Autologous Bone Marrow Transplantation

Recombinant GM-CSF Pharmacokinetics

Recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) is approved for use in patients undergoing high-dose chemotherapy and bone marrow transplantation. Administration of GM-CSF accelerates hematopoietic recovery after autologous bone marrow transplantation (ABMT) but also produces dose-related toxic effects. Pharmacokinetic studies of GM-CSF have indicated rapid drug elimination from serum after intravenous administration. Subcutaneous injection results in lower peak concentrations but sustained serum concentrations of >1 ng/mL.

We studied the relationship between the pharmacodynamics of GM-CSF and its clearance in 25 patients receiving continuous intravenous infusions of GM-CSF derived from a Chinese hamster ovary (CHO) expression system. Clearance increased in about half of the patients at the time of white blood cell (WBC) appearance in the periphery, suggesting increased WBC receptor binding of GM-CSF. Conversely, clearance decreased in patients experiencing renal dysfunction during the infusion (Figure 2). The percentage of GM-CSF dose found in 24-hour urine collections was substantially reduced in the latter group. A subset of patients who developed renal dysfunction also experienced significant hypotension. Rapidly increasing serum GM-CSF concentrations corresponded to the hypotensive episodes. Although these results offer useful information about clinical application of CHO-expressed GM-CSF, caution should be used in extrapolating to recombinant GM-CSF produced in other expression systems.

Characterization of Endogenous Cytokine Concentrations

A number of previous studies have individually evaluated endogenous cytokine concentrations of either macrophage colony-stimulating factor (M-CSF), interleukin-6 (IL-6), TNF-α, or erythropoietin. Reports have linked elevated endogenous M-CSF, TNF-α, and IL-6 to the development of major organ toxicity following ABMT, elevated M-CSF and IL-6 concentrations to several types of cancers, and elevated serum erythropoietin to anemia.

To better characterize the interactions of these cytokines, we studied the endogenous concentrations of all four proteins in 68 patients receiving ABMT. All patients received the same chemotherapy regimen and were transfused with red cells to maintain a hematocrit >42% until the time of leukocyte engraftment. Fourteen patients received G-CSF with peripheral blood progenitor cells (PBPC) in addition to ABMT; 17 received GM-CSF with PBPC; 17 received G-CSF with-
Concentrations of TNF-α, IL-6, and M-CSF were markedly lower among G-CSF/PBPC-treated patients than among other patients (Figure 3). In fact, although concentrations of IL-6 and M-CSF increased following ABMT in the other three treatment groups, peaking near day 12, concentrations decreased in the G-CSF/PBPC group. On day 12, concentrations of all three proteins were higher among patients treated without PBPC and those treated with GM-CSF. These observations are in accordance with in vitro studies indicating that recombinant GM-CSF can induce neutrophil and macrophage production of multiple cytokines. 35,37

Erythropoietin concentrations remained low throughout the course of therapy in most patients, indicating that our red blood cell transfusion regimen (transfuse for hematocrit <42%) was generally successful in reducing serum erythropoietin, compared with other reports, in which concentrations >100 mU/mL were evident on multiple days following transplantation. 32,31 However, concentrations increased >100 mU/mL in 6 of our patients late in the post-transplant period, despite maintenance of hematocrit >40% on most days. Hypotensive episodes occurred concurrently with erythropoietin elevation. We postulate that the etiology of these elevations was a reduction in renal blood flow.

On day 12, concentrations of all four cytokines were significantly higher in patients experiencing major organ toxicity after ABMT than in nontoxic patients (Table 1). To evaluate the sequence of events in patients developing toxicity, we defined elevated concentrations of cytokines as >7.5 pg/mL for TNF-α, >100 pg/mL for IL-6, >7 ng/mL for M-CSF, and >20 mU/mL for erythropoietin, as these concentrations were achieved on day 12 in a majority of toxic patients but in <25% of nontoxic patients. Using these criteria, we found elevated M-CSF concentrations in 15 of 21 patients before the first toxic event, with M-CSF elevation preceding the clinical manifestation of toxicity by a median of 7 days (P = 0.002) (Table 2). In 18 of 21 patients, TNF-α increased be-
fore the first toxic event. TNF-α release preceded toxic onset by a median of 3 days ($P = 0.001$).

Endogenous cytokine concentrations may also help account for the remarkable decrease in platelet transfusion requirements found in the G-CSF/PBPC group. Interleukin-6 is considered a possible platelet growth factor,$^{34,35}$ although recombinant M-CSF$^{44}$ or TNF-α$^{45,46}$ administration has been shown to produce transient, dose-limiting thrombocytopenia. We evaluated the effect of cytokine concentrations and treatment group on platelet transfusion requirements using multivariate regression (N = 67; R² = 0.440). The best predictor of platelet requirement was M-CSF ($P = 0.014$), followed by IL-6 ($P = 0.033$). High M-CSF and IL-6 both correlated with an increased demand for platelet transfusions. Interleukin-6 may be a marker for clinical problems or increased TNF-α production that results in thrombocytopenia. Alternatively, thrombocytopenic patients may have increased IL-6 production in response to low platelet count. Treatment group did not significantly affect platelets required in this analysis.

Effect of Endogenous TNF-α on G-CSF-Induced Hematopoiesis

Several in vitro studies have shown TNF-α to be an inhibitor of G-CSF-induced myeloid cell proliferation and have suggested that TNF-α downregulates G-CSF receptor expression on myeloid cells.$^{42,43}$ In our clinical evaluation of recombinant G-CSF, we noted a nonmonotone dose-response curve, with maximal myelopoiesis occurring at the middle dosage range (8 to 16 μg/kg/day) and leukocyte response diminishing at the highest dosage (64 μg/kg/day).$^{44}$ We also noted a positive relationship between dose and endogenous TNF concentrations ($P < 0.025$). To determine if endogenous TNF-α played a role in producing the nonmonotone dose-response curve, we examined the difference in WBC recovery in patients with detectable TNF-α ($>7.5$ pg/mL) on day 12 compared to those without detectable TNF-α.$^{45}$ We found that the median WBC on day 14 in patients with detectable TNF-α (N = 9) was only 800 cells/mm³, compared with 1,600 cells/mm³ in other patients (N = 27; $P < 0.05$). In addition, patients experiencing a significant decrease in myelopoiesis on days 8 to 14 were more likely to have detectable serum TNF-α on day 12 (7 of 13 patients) than were those with steadily increasing WBC (2 of 23 patients; $P = 0.0005$).

Endogenous Cytokines and IL-1-β Administration

Administration of recombinant IL-1-β following our high-dose che-

![Figure 3: Median post-transplant day 12 serum concentrations segregated on prescribed supportive-care regimen in 68 patients. M-CSF indicates macrophage colony-stimulating factor (ng/mL × 10); IL-6, interleukin-6 (pg/mL × 0.1); TNF, tumor necrosis factor-alpha (percent $>7.5$ pg/mL); and EPO, erythropoietin (mU/mL × 2.5). In addition, G-CSF indicates granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; and PBPC, peripheral blood progenitor cells.$^{34}$](image)

**Table 1. Cytokine Concentrations Obtained 12 Days After Autologous Bone Marrow Transplantation**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Patient Group</th>
<th>Median</th>
<th>Detectable (%)</th>
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<tbody>
<tr>
<td>TNF-α (pg/mL)</td>
<td>Nontoxic</td>
<td>ND</td>
<td>7/46 (15.2)</td>
</tr>
<tr>
<td></td>
<td>Toxic</td>
<td>9.91</td>
<td>5/21 (23.8)</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>Nontoxic</td>
<td>24.2</td>
<td>34/46 (73.9)</td>
</tr>
<tr>
<td></td>
<td>Toxic</td>
<td>160</td>
<td>19/21 (90.5)</td>
</tr>
<tr>
<td>M-CSF (ng/mL)</td>
<td>Nontoxic</td>
<td>3.53</td>
<td>44/46 (95.7)</td>
</tr>
<tr>
<td></td>
<td>Toxic</td>
<td>8.71</td>
<td>21/21 (100)</td>
</tr>
<tr>
<td>EPO (mU/mL)</td>
<td>Nontoxic</td>
<td>8.34</td>
<td>38/46 (82.6)</td>
</tr>
<tr>
<td></td>
<td>Toxic</td>
<td>20.8</td>
<td>18/19 (94.7)</td>
</tr>
</tbody>
</table>

*Sixty-eight patients are segregated based on the presence of renal and/or hepatic toxicity. Adapted and updated from Rabinowitz et al.$^{34}$ TNF-α indicates tumor necrosis factor-alpha; IL-6, interleukin-6; M-CSF, macrophage colony-stimulating factor; EPO, erythropoietin; and ND, not detectable.
Table 2. Sequence of Events in 21 Patients Experiencing Renal and/or Hepatic Toxicity After Autologous Bone Marrow Transplantation*  

<table>
<thead>
<tr>
<th>Event</th>
<th>No. Patients</th>
<th>Mean Days to First Occurrence</th>
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</thead>
<tbody>
<tr>
<td>MCSF &gt; 7 ng/mL</td>
<td>16</td>
<td>7.56 ± 3.76</td>
</tr>
<tr>
<td>EPO &gt; 20 mU/mL</td>
<td>15</td>
<td>8.80 ± 5.10</td>
</tr>
<tr>
<td>IL-6 &gt; 100 pg/mL</td>
<td>18</td>
<td>9.33 ± 4.12</td>
</tr>
<tr>
<td>TNF-α &gt; 7.5 pg/mL</td>
<td>20</td>
<td>9.55 ± 4.35</td>
</tr>
<tr>
<td>Cr &gt; 1.8 mg/dL</td>
<td>10</td>
<td>13.8 ± 4.08</td>
</tr>
<tr>
<td>TBLI &gt; 3.6 mg/dL</td>
<td>17</td>
<td>15.0 ± 4.01</td>
</tr>
</tbody>
</table>

*Adapted from Rabinowitz et al.*

| M-CSF indicates macrophage colony-stimulating factor; EPO, erythropoietin; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; Cr, serum creatinine; and TBLI, total serum bilirubin.

motherapy and ABMT regimen does not affect hematopoietic recovery. However, evaluation of endogenous cytokine concentrations has shown that IL-1β administration produced dose-dependent increases in endogenous cytokine concentrations. We measured concentrations of 6 different cytokines following ABMT in 16 patients receiving subcutaneous IL-1β at dosages of 4 to 32 ng/kg/day. We found that serum concentrations of 4 of the cytokines correlated significantly to IL-1β dose. Most remarkable were the high concentrations of G-CSF found on day 3, which were 3 times greater than endogenous G-CSF concentrations found in a matched set of patients receiving recombinant GM-CSF.

M-CSF in Fungemia

Murine studies have noted elevated serum M-CSF concentrations in animals inoculated with fungus. Clinical trials of M-CSF as an antifungal agent have resulted from these studies. To further our understanding of the role of M-CSF in fighting fungal infection in humans, we studied endogenous M-CSF serum concentrations in 20 ABMT patients with positive fungal blood cultures. Serum M-CSF increased more than threefold in a majority of patients at the time of positive culture, compared with concentrations obtained the previous week (median: 10.5 and 2.8 ng/mL, respectively; P = 0.001). These values were significantly higher than those in a set of patients—matched for chemotherapy regimen, cellular support, CSF given, and day of therapy—who did not have positive fungal blood cultures (N = 20; median: 3.1 ng/mL; P = 0.001).

Implications

Recombinant cytokines are a powerful tool in the continuing quest to reduce side effects and increase dose intensity of chemotherapy. In addition, these agents may prove useful in fighting infections and enhancing natural defense against tumor. However, a better understanding of the mechanisms of cytokine action is critical to effective application of these potent new drugs.

Studies of recombinant cytokine pharmacokinetics may reveal valuable information about clearance of these proteins. Our work, linking reductions in GM-CSF clearance to renal dysfunction, has resulted in close monitoring of patients in this setting for GM-CSF-related toxicity. In addition, pharmacokinetic studies have suggested that WBC receptor-mediated endocytosis may be a significant clearance mechanism for both GM-CSF and G-CSF.

The study linking endogenous TNF-α to reduction in G-CSF-induced hematopoiesis has enhanced our understanding of the mechanism of reduced G-CSF efficacy with higher post-transplant doses. This interaction also suggests that during periods of excessive cytokine secretion (eg, gram-negative sepsis), the efficacy of G-CSF therapy may, in theory, be altered.

Combinations of cytokines may be more effective than any single protein. The wide variety of possible combinations available is extremely exciting but also makes determination of the appropriate mixture of cytokines and timing of their administration difficult. Studies of patterns of endogenous cytokine release may help answer such questions. For example, our work, which relates high-serum M-CSF and IL-6 concentrations to an increased need for platelet infusions, suggests that the body may produce IL-6 in response to thrombocytopenia. Conceivably, a combination of recombinant G-CSF and IL-6 therapy could stimulate both granulocyte and platelet production. Further addition of M-CSF to such a regimen would not be recommended, as elevated M-CSF appears to block thrombopoiesis.

One of the most commonly discussed cytokine combinations is G-CSF and GM-CSF. Moderate or high doses of GM-CSF may induce TNF-α production, which could then down-modulate G-CSF receptors. In contrast, small doses of GM-CSF might synergize with moderate doses of G-CSF. Studies of cytokine kinetics are an efficient means of addressing such concerns.

Measurement of endogenous cytokine concentrations may also provide an indicator of biologic response to exogenously administered drug. A clinical trial on our bone marrow transplantation unit found no dose-related peripheral blood count response to IL-1β injection. One concern was that the drug could have been rendered biologically inactive before reaching its target receptors in the hematopoietic system. However, measurement of endogenous cytokines in these patients revealed a linear relationship between dose of IL-1β and concentrations of several cytokines. Therefore, IL-1β did interact with receptors in the hematopoietic system but failed to stimulate hematopoiesis as desired.

One of the most exciting new areas of cytokine research is the soluble receptors and receptor antagonists. As elevated TNF-α or IL-1β may gen-
erate some of the toxicities associated with chemotherapy or acute infection, molecules blocking the effect of these two proteins have been particular targets of study. Numerous therapeutic possibilities exist for reducing serum concentrations of these and other potentially harmful cytokines. Potential treatments include drugs such as pentoxifylline,17 ciprofloxacin,13 dexamethasone,18 monoclonal antibodies, soluble receptors, and receptor antagonists. However, cytokines such as TNF-α and IL-1β perform many important biologic functions, including aiding immune response, wound healing, tissue repair, and possibly some antimicrobial effects. The clinical effects of perturbing these processes are unknown.

Intervention with monoclonal antibodies or soluble receptors in specific instances is an appealing alternative to blocking production and/or action in a large variety of patients. We have noted that elevations in M-CSF concentrations tend to precede elevations in TNF-α concentrations in ABMT patients. Similar relationships may also exist in patients receiving less intensive chemotherapy or developing serious infections. Therefore, prospective measurement of a marker cytokine may enable early identification of patients who will have subsequent increases in toxic cytokine levels. Further studies of cytokine kinetics are crucial to exploring such a possibility.

In addition to providing insight into immune system regulation, measuring the concentrations of endogenous soluble receptors and receptor antagonists also suggests clinical applications. Treatment strategies mimicking natural mechanisms for minimizing the toxicity of TNF-α and IL-1β may be preferable to injection of drugs that inhibit their biologic activity in other ways. Appropriate dosing of recombinant soluble receptors or receptor antagonists depends on a thorough understanding of their normal endogenous regulation.

Conclusion

Studies of cytokine kinetics are a useful complement to clinical trials of recombinant growth factors. Measurement of the pharmacokinetics of the administered HGF is crucial to appropriate dosing and to understanding mechanisms of drug elimination. Measurements of secondarily released cytokines provide valuable information about the complete set of protein interactions that produce the drug's clinical effect. Moreover, secondarily released cytokines may be a useful measure of HGF biologic activity. Current work is venturing into progressively more complex areas, such as combination growth factor trials and studies of soluble receptors and receptor antagonists. Although the potential of such regimens appears extraordinary, full exploitation of their capacity may require understanding the interactions between a wide variety of cytokines.

References


