Hematopoietic effects of recombinant human interleukin-3 and interleukin-6
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Potential use of recombinant human interleukin-6 in clinical oncology


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Abstract

Recombinant human IL-6 (rhIL-6) is a pleiotropic cytokine with stimulatory actions on the hematopoietic system, the immune system and hepatocytes. Clinical interest in the use of this cytokine was raised because of its thrombopoietic properties and also because of its anti-tumor activity, which was shown in vitro and in the preclinical setting. Various studies showed that doses up to 10 µg/kg/d rhIL-6 before and after chemotherapy are tolerable and the most frequent side-effects encountered consist of flu-like symptoms. Furthermore, a consistent decrease in hemoglobin was reported during rhIL-6 treatment. This was probably due to hemodilution, although a change in ferrokinetics, may also at least partly, explain the anemia.

An evident increase of platelets has been observed in various studies. After chemotherapy, rhIL-6 seemed to hasten platelet recovery, without affecting platelet nadir. Preliminary data from studies investigating the value of rhIL-6 as an anti-tumor agent in renal cell carcinoma and melanoma reported low response rates, between 8 and 14%. The results of rhIL-6 in ameliorating chemotherapy induced bone-marrow depression, and especially thrombocytopenia, are promising and merit further phase-III-studies.
Cytokines comprise factors known to regulate the proliferation and differentiation of hematopoietic and lymphoid cells. Initially, the study of cytokines was hampered by low tissue concentrations resulting from low dosing schedules. Since the genes encoding for these cytokines were recognized with the aid of molecular biology, recombinant cytokines could be developed. This resulted in extensive laboratory and clinical research as sufficient supplies of these cytokines could now be guaranteed. Cytokines can be divided in several groups, such as, hematopoietic growth factors, interleukins, interferons, and tumor necrosis factors. Usually cytokines are glycoproteins which regulate various functions, several of them are pleiotropic and sometimes their functions overlap. This paper will focus on one of the cytokines, interleukin-6 (IL-6), and our experience with this cytokine in clinical oncology will be reviewed.

IL-6, a 26 kDa protein, is produced by T-lymphocytes, monocytes, fibroblasts, endothelial cells and keratinocytes [1]. The gene encoding for IL-6 is located on chromosome 7 and after the cloning of complementary DNA recombinant human IL-6 (rhIL-6) could be produced [2-6]. IL-6 is a pleiotropic cytokine with stimulatory actions on hematopoiesis as well as on the immune system [1] while it is also known to be involved in the acute phase response [7-10]. Special interest in the clinical use of rhIL-6 was raised because of its stimulatory effects on the hematopoietic system, and especially its effect on thrombocytopenia. Data from in vitro studies present evidence that rhIL-6 is a cytokine acting early in hematopoiesis, although most times in synergism with other cytokines [11-13]. Other studies revealed more late effects of rhIL-6, for example proliferative effects of rhIL-6 on megakaryocytes were shown in vitro and in vivo [14-16]. Animal studies also demonstrated also effects of rhIL-6 on the differentiation of megakaryocytes, and an increase in cell size and ploidy, were observed [14,17,18], which resulted in elevated peripheral platelet numbers, due to increased production [8,16,19-24]. In animals with bone marrow suppression due to prior whole body irradiation or chemotherapeutic drugs, rhIL-6 administration resulted in a faster recovery of platelets [23-28].
Another potentially interesting aspect of IL-6 is its antitumor capacity. Preclinical studies showed direct and indirect IL-6 mediated tumor cell destruction, which resulted in clinical trials assessing the value of this cytokine in the immunotherapy of cancer [29-31]. Contrary to these results tumor stimulatory effects also have been observed in vitro [32-34], which necessitates vigilance in clinical studies with respect to this unwanted effect. The two major aspects of rhIL-6 in clinical use will be focused upon, first its role in reducing chemotherapy induced bone marrow suppression and second studies evaluating its anti-tumor activity.

**RhIL-6 before chemotherapy.** Only a limited number of clinical studies have been published concerning the effect of rhIL-6 given before chemotherapy [35-36]. We have performed a phase I-II study to evaluate toxicity and hematological effects of rhIL-6 administered prior to chemotherapy [35]. Twenty patients with breast cancer or non small cell lung cancer (NSCLC) received rhIL-6 at six different dose levels, i.e., 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 μg/kg body weight/day (μg/kg/d), with at least three patients per dose step. RhIL-6 was given intravenously (iv) on the first day, followed by subcutaneous (sc) administration the following six days. One week after cessation of rhIL-6 administration chemotherapy was started. Flu-like symptoms, including fever, headache and myalgia were frequently observed at all dose levels. From 2.5 μg/kg/d rhIL-6 onwards, nausea, as well as reversible increases in liver function tests and anemia occurred. Significant increases in the mean number of platelets from the pooled data from all patients (0.5-20.0 μg/kg/d rhIL-6) was observed during and after rhIL-6 administration (Figure 1). Furthermore, the mean number of leukocytes, neutrophils and monocytes showed significant elevations compared to baseline values during rhIL-6 administration, this effect was still present at the moment chemotherapy was started. Other interesting features were a decrease in serum cholesterol and a sharp increase in acute phase proteins during rhIL-6 administration. Flu-like symptoms were manageable with acetominophen administration up to the 10 μg/kg/d rhIL-6 dose level. Nausea could be controlled with metoclopramide. No dose limiting toxicity was encountered in this study.
Weber et al. treated patients with 3.0, 10.0 and 30.0 μg/kg/d rhIL-6 sc for seven days (n=11), with comparable results as those obtained by van Gameren. They observed flu-like symptoms and fatigue in all patients. Furthermore a dose dependent anemia was observed in this study. At 30.0 μg/kg/d rhIL-6, dose limiting toxicity occurred in two patients, i.e., cardiac dysrhythmia and severe hepatotoxicity. Significant increases in platelet numbers were observed for 10.0 and 30.0 μg/kg/d rhIL-6, without changes in megakaryocyte numbers as shown in bone marrow biopsies. These increases reached maximum values one week after discontinuation of rhIL-6. Dose dependent increases in acute phase proteins were observed during rhIL-6 administration [36]. Another study by Weber et al. showed similar results [37]. Eighteen patients were treated with one-hour iv infusions of rhIL-6 doses of 0.3 to 30.0 μg/kg three times daily for seven days. Fever and chills were observed frequently and dose limiting toxicity was reached at 30.0 μg/kg every eight hours, consisting of reversible neurotoxicity. A reversible anemia was seen at lower doses. Platelet counts, white blood cell counts and acute phase proteins were substantially elevated. RhIL-6 (1, 2.5, 5 and 10 μg/kg/d) administered sc to children was tolerable up to the highest dose level, with fever, chills and fatigue experienced by all of the patients (n=10). Significant increases in platelet counts were observed, ranging from 27 to 238% compared to base-line levels. Bone marrow examination at day one and 14 revealed an increase in ploidy, without changes in the number of megakaryocytes [38].

The results in these studies are to a large extent in agreement with one another. Based on these studies a rhIL-6 dose of 20 μg/kg/d seemed feasible, with flu-like symptoms and anemia being the most frequently observed side-effects. An increase of platelets occurred during rhIL-6 administration, with maximum values approximately one week after discontinuation of rhIL-6 [35]. Mean platelet volume

![Figure 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 (**).](image-url)
decreased during rhIL-6 treatment [35], with increases in ploidy after 14 days [38] and without changes in the number of megakaryocytes [36]. Effects were also observed in other cell lineages, neutrophils, monocytes and lymphocytes tended to increase during rhIL-6 administration. These lymphocytes were phenotypically similar to T-cells, NK-cells and cells expressing the IL-2 receptor [35].

RhIL-6 after chemotherapy. The study performed by van Gameren et al. was followed by a second part, in which rhIL-6 was administered after chemotherapy [39]. Nineteen patients were treated with the same dose of rhIL-6 as given prior to pre-chemotherapy. Chemotherapy was given at day one, which was two weeks after the start of rhIL-6 administration in the pre-chemotherapy period and consisted of mitoxantrone (10 mg/m²) and thiotepa (40 mg/m²) both given iv as a bolus on a three-weekly basis, a total of six cycles were scheduled. RhIL-6 (sc) was started at day five and given for ten days. A total of 48 and 45 cycles were evaluable for toxicity and hematological effects, respectively. Just as in the pre-chemotherapy period, flu-like symptoms were observed frequently, which responded well to acetaminophen administration. Three patients experienced worsening of their pre-existing dyspnea, not exceeding WHO grade 2. Nausea occasionally followed by vomiting during rhIL-6 treatment was observed in four patients (seven cycles) and was successfully treated with metoclopramide. Nearly all patients experienced erythema at the rhIL-6 injection site, which resolved after discontinuation of the injections. Dose limiting toxicity was reached at 20 mg/kg/d rhIL-6 due to WHO grade 3 nausea and vomiting in one patient and WHO grade 3 fever concurrent with flu-like symptoms in another patient. The last patient discontinued the study prematurely due to severe flu-like symptoms.

For those at 10.0 and 20.0 mg/kg/d rhIL-6 a faster platelet recovery was observed, compared with the lower dose levels. The platelet nadir occurred significantly earlier for those at the two highest doses, but the extent did not differ. No differences were found for the frequency of WHO grade 4 thrombocytopenia among the six dose levels; however, the two patients who received platelet transfusions were treated at the 1.0 and 2.5 mg/kg/d rhIL-6 dose level. No different effects were observed on the
leukocyte number between the six dose levels. Comparable results were obtained by D’Hondt et al. who administered rhIL-6 prior and after chemotherapy [40]. In the pre-chemotherapy period a dose related increase in platelets were observed, and after chemotherapy a faster recovery platelets occurred [40]. Preliminary data from other studies, reporting the effects of rhIL-6 after chemotherapy, showed no dose limiting toxicity up to 10 µg/kg/d rhIL-6 [40-42]. The profile of the observed side effects in all of these studies was comparable.

Lestingi et al. (n=27) observed an amelioration of the chemotherapy induced thrombocytopenia, with higher nadir levels after rhIL-6 when compared with cycles without rhIL-6 support [41]. In contrast to our results, they found a better response at 1 µg/kg/d than at 10 µg/kg/d rhIL-6. Another study however, revealed no differences in platelet nadir compared to historical controls when rhIL-6 (2.5 µg/kg/d) was administered to melanoma patients (n=19) following chemotherapy [43]. A faster recovery of platelets was observed by Hamm et al. (n=36) at a dose level of 5 µg/kg/d rhIL-6, co-administered with G-CSF, but this effect seemed to diminish in a second cycle [42]. RhIL-6 (5.0 µg/kg/d) following high-dose chemotherapy for autologous bone marrow transplantation also resulted in accelerated platelet recovery compared to controls [44].

In summary, most studies reported no effects on platelet nadir, but there was a trend toward a faster recovery of platelets. This effect on recovery is probably not due to increased proliferation of megakaryocytes, but result of increased differentiation of these cells and subsequent thrombopoiesis. Whether this quicker recovery alone will ultimately result in the ability to use an increased chemotherapy dose intensity, remains questionable. Phase III studies are needed to confirm the effects of rhIL-6 after chemotherapy. Recently, Tritarelli et al. reported a synergistic stimulation of hematopoiesis in mice when rhIL-6 was combined with rhIL-1β, furthermore the recovery of myeloid cells was enhanced after cyclophosphamide treatment [45]. Based on these data, combination of rhIL-6 with rhIL-1β may also be considered for clinical trials. Whether the use of rhIL-6 alone or in combination with other cytokines will ultimately result in increased dose intensity and consequently in prolonged survival of cancer patients remains to be established.
**RhIL-6 as antitumor agent.** Our department participated in a multicenter phase II trial in which antitumor effects of rhIL-6 were assessed in patients with metastatic renal cell carcinoma [46]. To be eligible patients had to have evaluable disease. RhIL-6 was given daily sc for six weeks in a fixed dose of 150 μg per day. Tumor response was evaluated after six and after ten weeks. The results were analyzed for two cohorts, patients who had not received prior immunotherapy and those who had. A total of 35 patients were entered and 29 were evaluable for response, of which 19/29 patients had received prior immunotherapy. In those who had received prior immunotherapy two partial responses were observed both occurring very late, one after six weeks and one after as many as 18 weeks. Stable disease occurred in seven and four patients with and without prior immunotherapy respectively. Progressive disease was observed in 10 patients who had not, and six who had received prior immunotherapy. Objective antitumor response was observed in 10% in the non-pretreated group. Toxicity consisted of fever and nausea, furthermore anemia and reversible increases in alkaline phosphatase and gamma glutamyl transferase was observed during rhIL-6 therapy. Sznol et al. reported one complete remission in 13 patients with metastatic malignant melanoma treated with daily one-hour iv infusion of 100 μg/kg/d (!) rhIL-6 for 5 out of 7 days the first two weeks; this schedule was repeated after four weeks [47]. Compared to other studies a considerable rhIL-6 dose was administered, however, toxicities were mild and this regimen was fairly well tolerated by the majority of the patients. A phase II trial for patients with metastatic renal cell carcinoma (n=14) of 120 hour continuous iv administration of rhIL-6 repeated every three weeks resulted in a modest tumor response, one patient had a partial response and one had stable disease, the others were progressive [48]. In this study 30 μg/kg/d rhIL-6 was considered to be the maximum tolerated dose, with atrial fibrillation occurring in three patients, two of these patients had electrocardiographical signs of ischemia, which spontaneously resolved without signs of myocardial infarction.

In summary, these preliminary results reveal low response rates, between 8 and 14%. The mechanism of action is still unclear. Direct cytotoxicity and CTL (cytotoxic T-lymphocyte) mediated mechanisms have been suggested [29,31]. Others proposed a more prominent role for activated natural killer (NK) and lymphokine activated killer cells (LAK) as a means of IL-6 induced tumor kill, based on in vitro data [49-52].
However, these data were in contrast with those obtained by Scheid et al., who studied the immune function of cancer patients receiving rhIL-6. They showed suppression of NK- and LAK-activity at rhIL-6 doses exceeding 2.5 μg/kg/d [10]. Furthermore, suppression of NK-activity in mice was hypothesized by Tanner et al. who reported tumorigenicity of EBV immortalized lymphoblastoid cells in the presence of IL-6 [53]. In certain hematological malignancies, i.e., multiple myeloma and Castleman’s disease, there is strong evidence that endogenous IL-6 may function as a growth stimulating factor for malignant plasma cells [32,54-56]. In vitro, stimulatory actions of rhIL-6 on solid tumors have also been observed [33,34], but clinical data concerning stimulatory actions of rhIL-6 are scarce. Recently, Ravoet et al. presented two patients with transient accelerated growth of tumor during treatment with rhIL-6, followed by a reduction in tumor size after discontinuation of rhIL-6 treatment [57]. One patient was evaluated with liver metastases from ovarian adenocarcinoma, the other with lung metastases from renal cell carcinoma. It is as yet unclear whether this was due to a temporary increase in tumor size or due to the disappearance of an induced lymphocyte infiltration in the tumor [57]. This raises the as yet unresolved question whether the use of hematopoietic growth factors, and rhIL-6 in particular, as a means of increasing chemotherapy dose intensity or as an antitumor agent, could act as a stimulator of certain solid tumors.

RhIL-6-induced anemia. In preclinical as well as in clinical studies anemia has been observed during rhIL-6 administration [21,24,35-37,45,58], necessitating blood transfusions in a number of cases [46]. Recent reports demonstrated the normochromic and normocytic character of this anemia [35-37,59]. A rhIL-6 dose related decrease in hemoglobin concentration was observed within three days of rhIL-6 administration by van Gameren et al. (Figure 2), associated with a decrease in serum iron without signs of hemolysis or bone marrow failure and normal bone marrow iron stores, folate, and vitamin B₁₂ despite increased erythropoietin levels [35]. In patients treated with chemotherapy and rhIL-6 also a significant decrease was observed in hemoglobin concentration [39,41,42]. The rapid occurrence of this anemia points to either changes in plasma volume or sequestration in the spleen,
once hemolysis is excluded. Based on radioisotope dilution assays, both Atkins et al. and Nieken et al. independently showed that rhIL-6 associated anemia was primarily due to increases in plasma volume [58,59]. Others reported a similar mechanism, for anemia associated with rhIL-11 administration, due to an increased plasma volume associated with urinary sodium retention [60]. Additional factors might also be involved, because van Gameren et al. [35] and Weber et al. [36] both observed a rapid drop in serum iron during rhIL-6 treatment. Furthermore, stainable iron was absent from bone marrow biopsies in some patients. Kobune et al. recently demonstrated a rapid hypoferremia during rhIL-6 treatment in rats [61], which was caused by mobilizing transferrin-bound serum iron to hepatocytes. In addition, they found increased ferritin and decreased transferrin synthesis. This change in ferrokinetics may be in part responsible for the observed anemia.

Conclusions

Based on promising in vitro and preclinical experiments, clinical studies were performed to evaluate the hematopoietic and anti-tumor effects of rhIL-6. Thus far, only data from phase I/II studies are available. Stimulatory activity on platelets of rhIL-6 was clearly established in studies where the drug was given before chemotherapy. After chemotherapy rhIL-6 administration resulted in a faster recovery, without affecting platelet nadir. In contrast to the aim of these studies, which was to reduce in bone marrow toxicity, anemia was evident which was most likely due to hemodilution. It is debatable whether this side effect outweighs the
benefits of stimulation of platelet recovery. Therefore, phase III trials are needed to assess the value of rhIL-6 in reducing chemotherapy induced bone marrow depression and especially thrombocytopenia, in relation to its side effects. Based on in vitro data, the combined use of rhIL-6 and rhIL-1β in ameliorating chemotherapy induced bone marrow depression may also be considered. Available preliminary data concerning anti-tumor activity of rhIL-6 showed that it can on its own have anti-tumor activity. The exact response rates for potentially interesting tumor types are not yet available, but tend to be low. Special care will have to be taken concerning the potential tumor promoting effect of rhIL-6 in certain tumor types. This review showed that IL-6 is an enigmatic pleiotropic cytokine, that will remain the subject of fundamental and clinical studies for many years to come.

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