Induction of Interleukin 1 Receptor Antagonist after Interleukin 1 Therapy in Patients with Cancer

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ABSTRACT

The interleukin 1 receptor antagonist (IL-1ra) is a naturally occurring molecule that shares homology with IL-1α and IL-1β and binds competitively to IL-1 receptors. Serum concentrations of IL-1ra were measured by ELISA in patients enrolled in Phase I clinical trials of IL-1α and IL-1β given by 15-min infusion. Pretreatment levels of IL-1ra were <1500 pg/ml in some patients within 1 h of completing the IL-1 infusion. By 2 h after infusion, serum levels of IL-1ra had increased dramatically, and they remained stable 4 h after infusion. There was evidence that peak IL-1ra levels were IL-1 dose dependent. Seven patients treated with IL-1α had IL-1ra levels that exceeded 1 µg/ml. In contrast, serum levels of IL-1 declined rapidly and were undetectable 1 h after completing IL-1 infusion in most patients receiving <1.0 µg/kg. IL-1ra levels remained slightly elevated over pretreatment values in serum obtained 18–24 h after IL-1 infusion, but there was no evidence for progressive accumulation over repeated days of therapy. A similar pattern of IL-1ra elevation was observed after the last IL-1 infusion on day 6. This study shows that cancer patients produce 2 to >6 log incremental increases in IL-1ra rapidly following treatment with IL-1, a response that has implications for the design of future clinical trials with IL-1 and with agents thought to induce IL-1 production.

INTRODUCTION

IL-1β is a cytokine with pleiotropic effects that include costimulation of T lymphocytes (1, 2), myeloprotective activity against radiation and chemotherapeutic agents (3, 4), and direct in vitro antitumor activity (5, 6). These properties, among others, suggest a potential role for IL-1 in the treatment of cancer and have led to the performance of clinical trials. Phase I trials of IL-1α and IL-1β conducted at the Biological Response Modifiers Program have demonstrated that the inpatient MTD for both agents, when given daily for 7 days by 15-min i.v. infusion, is 0.3 µg/kg (7, 8). The dose-limiting toxicity was hypotension. Dose-related increases in peripheral WBC counts, primarily in neutrophils and neutrophilic bands, occurred during therapy, followed by a delayed increase in platelet counts after therapy. Over the 7 days of IL-1 treatment, patients showed evidence of tachyphylaxis in WBC response. Tachyphylaxis also has been reported for certain acute toxicities, such as hypotension in patients enrolled in a Phase II trial of IL-1α plus indomethacin in melanoma (9). In these three clinical trials, the overall antitumor response to daily bolus IL-1 therapy has been <5%.

IL-1α and sIL-1Rs (10–12) have been implicated in regulating IL-1 activity. IL-1α binds to both the type I (13, 14) and type II (15–17) IL-1Rs competitively and blocks the bioactivity of IL-1, which is mediated through type I receptors (18) both in vitro (17, 19) and in vivo (20, 21). However, IL-1α is unable to induce signal transduction after interaction with IL-1R and has no clear agonist properties (13). Demonstration of IL-1R blockade has required generally a 10–1000-fold molar excess of IL-1α, and this requirement is thought to reflect the need for only a low percentage of receptor occupancy for IL-1 to mediate its biological effects (22).

The potential of IL-1α to limit IL-1-mediated injury in chronic inflammatory disease and life-threatening situations such as septic shock has led to clinical trials. A Phase I clinical trial (23) established that IL-1α can be given at doses >10 mg/kg without toxicity. This study has been followed by clinical trials in patients with septic shock (24), steroid-resistant acute graft-versus-host disease (25), and rheumatoid arthritis (26). Although there was no overt survival benefit in the septic shock patients, there was evidence for clinical improvement in some patients with graft-versus-host disease and arthritis.

In this study, we show that within 2–4 h of IL-1 treatment, there is a dose-dependent increase in serum levels of IL-1α, reaching levels >1 µg/ml in some patients.

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PATIENTS AND METHODS

Subjects. Seventy patients with advanced cancer were enrolled in separate Phase I trials of IL-1α (Dainippon Pharmaceutical, Co., Ltd., Osaka, Japan) and IL-1β (Syntex Research, Palo Alto, CA). The majority of patients had been diagnosed with colorectal cancer, melanoma, or breast cancer. All patients gave written informed consent to participate in these clinical protocols, both of which were approved by the Institutional Review Boards of the Clinical Oncology Program of the National Cancer Institute and the Frederick Cancer Research and Development Center.

Clinical Trials. The protocol design of both Phase I clinical trials was identical. Details of the protocol design and clinical aspects of the IL-1α trial have been published (7), and results of the IL-1β trial have been presented in abstract form (8). Briefly, IL-1 was given by 15-mm i.v. infusion daily for 7 days with day 0 being the first treatment day. Cohorts of three to six patients received one of the following doses of IL-1: 0.01, 0.03, 0.1, 0.3, or 1.0 μg/kg. After reaching a MTD of IL-1 alone, additional patients were treated with IL-1 on the same schedule plus p.o. indomethacin given every 8 h. Dose escalation was performed until an MTD of the combination was reached. Sera were separated and frozen at −80°C.

ELISA for IL-1ra. Serum levels of IL-1ra were measured by ELISA (R&D Systems, Inc., Minneapolis, MN). Due to limitations in the quantity of serum available for testing, initial testing of most samples was performed using serum diluted 1:2. In individual assays, the lower limit of the standard curve used for data analysis, including the serum dilution factor, ranged from 94 to 375 pg/ml. For the purpose of this report, serum samples with IL-1ra levels ≥375 pg/ml were assigned an arbitrary value of 100 pg/ml. The average level of IL-1ra in normal serum, as reported by the manufacturer, is 201 pg/ml, with values >1000 pg/ml observed in ~1% of normals tested.

ELISAs for IL-1α and IL-1β. Serum levels of both IL-1α and IL-1β were determined by ELISA. IL-1α ELISA kits were obtained from Endogen (Boston, MA), and the limit of sensitivity of the assay as performed for this study ranged from 25 to 75 pg/ml in individual assays. IL-1β ELISA kits were obtained from Cistron Biotechnology (Pine Brook, NJ), and the lowest concentrations of standard used for analysis were 15.6–50 pg/ml in individual assays. Normal serum levels of IL-1α and IL-1β are reported to be below the respective detection limits of these assays.

Statistical Methods. Descriptive statistics, repeated measures ANOVA, parametric (Tukey) and nonparametric (Jonckheere) trend tests (27), and pharmacokinetic analyses were performed for these data. Most analyses were performed on log-transformed data to satisfy homogeneity assumptions.

RESULTS

IL-1α Pharmacokinetics. IL-1α levels were measured in serum samples obtained beginning 5 min after completing infusion on day 0 and/or day 6 in six patients, three each treated at 0.3 and 1.0 μg/kg, respectively. Peak serum levels of IL-1α ranged from 95 to 435 pg/ml in patients treated at the 1.0-μg/kg dose. IL-1α levels declined rapidly and in two patients were undetectable, <25 pg/ml, within 1 h of completing infusion and in the third patient fell below the detection limit of the assay 3 h after infusion. Only one patient treated at the 1.0-μg/kg dose completed the scheduled 7-day course of therapy, and on the last day of infusion, IL-1α levels were maintained at steady state levels of 50–70 pg/ml throughout the 4-h monitoring period. At the 0.3-μg/kg dose, IL-1α was detected in only a single serum sample obtained on day 6 at a level of 48 pg/ml. None of the other day 0 or day 6 samples from these patients contained measurable levels of IL-1α.

Clearance of IL-1β from serum was evaluated in 37 of 42 patients and included patients treated at all IL-1β dose levels. IL-1β was not detected in the serum of patients before treatment. There was a dose-dependent increase in peak serum IL-1β levels after therapy, as measured by Jonckheere’s nonparametric test for trend (P = 0.0001; Table 1). Serum obtained from patients treated with IL-1β at doses ≥0.3 μg/kg contained <100 pg/ml IL-1β 1 h after completing the infusions. Four h after infusion, only three patients had detectable IL-1β, and in each case, the concentration was at the lower detection limit of the assay. In the two patients treated with 1.0 μg/kg IL-1β, serum levels were 630 and 290 pg/ml, respectively, 1 h after therapy and 72 and 88 pg/ml, respectively, 4 h after therapy. IL-1β remained undetectable (20 pg/ml) in the serum of one of these patients 24 h after infusion. Attempts to model the pharmacokinetic elimination of IL-1 were generally unsuccessful, because IL-1β serum levels were undetectable in the majority of patients within min of completing therapy. Those few patients whose samples did show a distinct clearance pattern had apparent α half-lives for IL-1β of <10 min. Insufficient time points were collected to establish β half-lives.

IL-1ra. Combining data from the two separate clinical trials, the mean patient pretreatment IL-1ra concentration was 453 [range, 100 (below the assay threshold)–1338] pg/ml. A
obtained 18–24 h after the first IL-1 dose, suggesting that IL-1ra levels do not accumulate with repetitive therapy. Induction of IL-1ra were elevated on day 6 and were comparable to levels repeated after the seventh dose of IL-1. Measurements were further (Table 2).

Serum IL-1ra levels after the first dose of IL-1 in 12 patients tested 1 and 4 h after IL-1 therapy. Samples with activity below the sensitivity of the assay were assigned a value of 100 pg/ml. Three patients had IL-1ra levels 1 h after therapy that exceeded the upper detection limits of the individual assays, 2, 3, and 6 ng/ml.

Fig. 1  Serum IL-1ra levels after the first dose of IL-1β in 12 patients tested 1 and 4 h after IL-1 therapy. Samples with activity below the sensitivity of the assay were assigned a value of 100 pg/ml. Three patients had IL-1ra levels 1 h after therapy that exceeded the upper detection limits of the individual assays, 2, 3, and 6 ng/ml.

Changes in IL-1ra levels in patients treated with IL-1α were similar to those of patients treated with IL-1β: marked increases were observed in samples obtained 4 h after therapy on both day 0 and day 6 (Table 3). Increasing levels of IL-1ra were observed with increasing IL-1 doses (P = 0.0001). Seven patients, six of whom were treated with ≥0.3 µg/kg IL-1α, had IL-1ra levels that exceeded 1.0 µg/ml. These serum values should be considered approximations, because serum dilutions of 1:1,000–1:10,000 were required for the sample to fall within the standard curve of the assay. Direct comparisons of day 0 and day 6 IL-1ra levels could be made for only seven patients, all of whom had higher IL-1ra levels after the first IL-1 dose. Indomethacin treatment had no apparent effect on IL-1ra levels induced by IL-1 therapy.

DISCUSSION

IL-1-mediated inflammation is regulated by the balance of IL-1 with its inhibitors, which include IL-1ra and sIL-1R. This regulation is made more complex, because the type I sIL-1R also binds to IL-1ra strongly (12, 28). In animal models of inflammatory diseases, IL-1ra reduces the incidence and severity of inflammation (21, 29, 30). In healthy humans, the endotoxin-induced increase in circulating neutrophils was attenuated by coinfusion of endotoxin with IL-1ra (31). In humans, the balance of IL-1 to IL-1ra in synovial fluid has been correlated with the time to recovery from episodes of Lyme arthritis (32). In addition, a decrease in the IL-1ra:IL-1 ratio of intestinal mucosal cells and mucosal biopsies relative to controls was observed in patients with Crohn’s disease and ulcerative colitis and correlated with clinical severity (33).

In our trials, IL-1 therapy induced predictable, dramatic increases in serum IL-1ra. These increases were detected even after treatment with the lowest IL-1α dose administered, 0.01 µg/kg, a dose that had no effect on patients’ WBC counts but did induce grade I and II toxicities (7). These results show that administration of small amounts of cytokine can overcome constitutive levels of IL-1 antagonists. IL-1ra levels peaked within 2–4 h, and by 4 h, all but one patient tested had IL-1ra levels that required serum dilution to fall on the standard curve of the ELISA. In patients treated at IL-1 doses ≤0.1 µg/kg, the levels of IL-1ra induced and the time courses of induction were comparable to those reported previously by Bargetzi et al. (34) for six patients treated with IL-1β at doses of 0.05–0.1 µg/kg and by Tilg et al. (35) for four patients treated with IL-1α at a dose of 0.03 µg/kg. However, our results expand on these
reports by including data obtained from a significantly larger number of patients and from patients treated with higher, more toxic doses of IL-1. It was only at these higher IL-1 doses that IL-1 levels were detectable in serum, allowing both IL-1 and IL-1ra levels to be monitored.

At IL-1 doses ≥0.1 μg/kg, peak serum levels of IL-1ra were consistently >100 ng/ml and in some patients exceeded 1.0 μg/ml, levels comparable to peak levels attained in patients treated with 1.0 mg/kg recombinant IL-1ra (23). In contrast, in most patients, IL-1 was undetectable in serum 2–4 h after infusion and was <1 ng/ml even in patients treated with IL-1 doses above the MTD. Therefore, we consistently observed IL-1ra at a >100-fold molar excess 2–4 h after IL-1 infusion. However, immediately after IL-1 infusion, serum concentrations of IL-1B reached levels comparable to, and in some cases in excess of, the concentration of IL-1ra, a condition favoring the interaction of IL-1 with its receptors.
Comparison of IL-1ra levels achieved on days 0 and 6 shows that patients maintain the ability to produce high levels of IL-1ra in response to IL-1. Data from seven patients whose IL-1ra levels were measured on both day 0 and day 6 suggest that patients have lower peak IL-1ra levels on day 6, which is also consistent with previous reports (34, 35).

Elevated levels of serum IL-1ra have been reported in volunteers receiving injections of endotoxin (36, 37), patients with sepsis (38), surgical and trauma patients (39, 40), and patients receiving high-dose IL-2 (41) or IFNs (42). IL-1ra levels reported in these studies were comparable to levels we observed in patients receiving IL-1α doses <0.1 µg/kg. The only report of IL-1ra levels comparable to those of patients treated with IL-1 at doses ≥0.3 µg/kg was in subjects who received recombinant IL-1α (23).

Cancer patients can mount a vigorous IL-1ra response after IL-1 challenge. The molar excess of IL-1α observed in these trials exceeds the values reported to achieve receptor blockade and to modulate IL-1-mediated biological effects in vitro. Although serum levels of IL-1α had declined by the time of the next daily IL-1 injection, some degree of IL-1ra-mediated receptor blockade may remain in effect at the time of subsequent IL-1 doses. Although not measured in this study, the inflammatory response to IL-1 includes induction of soluble tumor necrosis factor receptors (34, 35), and sIl-1Rs also may be induced. There are low levels of constitutive sIl-1R in synovial fluid, and the type II sIl-1R is elevated in inflammatory synovial fluid (12) and in the serum of patients with sepsis (43). The combined effects of these regulatory mechanisms may be to reduce the biological activity of IL-1 despite continued therapy.

We hypothesize that induction of IL-1ra contributes to the tachyphylaxis in hematological effects (7, 8) and toxicity (9) reported after daily bolus IL-1 therapy and propose that future clinical trials of IL-1 should be designed to reduce the potential impact of IL-1R blockade. The advantage of bolus infusion regimens is that peak serum IL-1 concentrations are achieved before induction of antagonists, thereby favoring the interaction of IL-1 with its cellular receptors. Because IL-1 signal transduction is rapid, the delayed production of excess IL-1ra or sIl-1R would not prevent maximal IL-1-mediated biological effects after the first IL-1 dose. However, IL-1 injection may generate a receptor blockade that prevents optimal stimulation with subsequent doses. The low serum levels of IL-1ra present 24 h after an IL-1 dose may not reflect the level of receptor blockade persisting in the tissues. Thus, it would be of interest to examine the biological effects of twice or thrice weekly bolus administration of IL-1. Such schedules would provide a greater opportunity for IL-1ra and sIl-1R clearance between doses and would provide more favorable conditions for detecting maximal IL-1-mediated biological effects.

The induction of IL-1ra by IL-1 illustrates an important principle for the development of biological products as antitumor agents. The design of a schedule of IL-1 administration based on pharmacological principles (e.g., target serum level and clearance rate) is likely to look very different from an optimal regimen that takes into account the physiological homeostatic mechanisms that resist unopposed immune stimulation. Biological agents must be developed as therapeutic agents, with an understanding of the physiological processes regulated by the agents. For the vast majority of biological agents, our understanding of human immune physiology is too rudimentary and incomplete to predict their in vivo effects rationally. We must abandon the cytotoxic drug development paradigm or risk missing important effects of biological agents and throwing them away erroneously.

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