Altered Tryptophan and Neopterin Metabolism in Cancer Patients Treated with Recombinant Interleukin 2

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ABSTRACT

Immune stimulation or interferon administration induces indoleamine 2,3-dioxygenase and GTP cyclohydrolase activity in humans, resulting, respectively, in tryptophan degradation to kynurenine and in neopterin production. To determine if similar effects result from interleukin 2 (IL-2) administration, plasma tryptophan and urinary kynurenine and neopterin were measured in patients undergoing a phase 1 toxicity trial of recombinant IL-2 given by daily bolus or continuous i.v. administration for 7 days at doses of 1 x 10^6 to 1 x 10^7 units/ml/day. Significant dose-dependent decreases in plasma tryptophan levels and corresponding increases in urinary kynurenine and neopterin were observed. These metabolic effects of IL-2 are probably mediated by induction of γ-interferon production, although elevated levels of γ-interferon were not found in the sera of these patients. In view of the indispensable role of tryptophan in synthesis of protein, niacin, and serotonin, we suggest that some of the toxic side effects may be the result of loss of tryptophan.

INTRODUCTION

Infection, lipopolysaccharide administration, or interferon treatment results in the induction of a recently described enzyme, IDO (EC 1.13.11.17), in a variety of nonhepatic tissues (1-3). This enzyme is distinct from the liver tryptophan dioxygenase enzyme in its tissue distribution, molecular weight, immunogenicity, requirement for superoxide anion, and inducibility. Like the liver enzyme, it results in conversion of L-tryptophan to formylkynurenine and subsequent metabolites of this pathway.

We recently reported that human recombinant IFN-γ induced tryptophan degradation in vitro in human cell cultures and in vivo in patients in phase I clinical trials (4-6). Unlike IFN-γ, α- and β-interferon did not induce tryptophan degradation in vitro in human lung or bladder-derived cells (4) but type I interferons induced IDO in cultures of mixed peripheral blood mononuclear cells (7) and β-interferon administration resulted in decreased serum tryptophan and increased urinary excretion of KYN (8). These increases in tryptophan catabolism are undoubtedly the result of the induction of IDO (1-3). IDO is an extrahepatic enzyme, distinct from liver tryptophan dioxygenase, which opens the indole ring of tryptophan, and to a lesser extent of serotonin and certain other indoles, using superoxide anion as a required oxygen source (3, 9). IDO is not induced by conditions which induce liver tryptophan dioxygenase (1-3) and, under conditions which induce IDO, the liver enzyme levels are significantly reduced (3). In view of the requirement of IDO for superoxide anion, it may be significant that the induction of xanthine oxidase, a potential source of superoxide anion, is another reported effect of IFN-γ (10).

Production and excretion of NEO, a metabolite of GTP, is also increased in some cancer patients by allogeneic immune stimulation and by IFN or IL-2 administration (8, 11-15). This increase in NEO results from induction of GTP cyclohydrolase (EC 3.5.4.16), which initiates NEO synthesis in monocytes/macrophages. Although a direct relationship between NEO and tryptophan metabolism is not known, we have observed that stimulation of tryptophan metabolism and of NEO production after IFN-γ are correlated (8).

The effect of IL-2 on IDO activity or on tryptophan metabolism in vivo has not been reported, but since IL-2 stimulates production of IFN-γ in vitro, it might be expected that IL-2 would also indirectly induce IDO and GTP cyclohydrolase in vivo. The present study was done to assess the effects of IL-2 on tryptophan metabolism and NEO excretion in cancer patients receiving IL-2 in a phase I clinical trial and to evaluate the utility of tryptophan and NEO measurements as indices of biologically effective doses of IL-2.

MATERIALS AND METHODS

IL-2 Patients. Patients were those entered into approved protocols of the University of Wisconsin Clinical Cancer Center for a phase I trial of IL-2 toxicity. Informed consent was obtained. In accordance with phase I (toxicity) protocols, a variety of cancer patient types were eligible for this study (16). Patients in this study included three renal cell, two breast, three lung, three melanoma, and one each of leiomysarcoma, colon, rectal, pancreatic, parotid gland, synovial sarcoma, poorly differentiated nodular lymphoma, and thyroid tumors. Further details of this clinical trial have been reported (16). Briefly, patients had biopsy-proved malignancy which was surgically incurable and for which other standard therapies failed or were not known to be effective. Other criteria for eligibility included life expectancy of greater than 4 months, age greater than 18 years, blood and urine chemistry within specified limits, more than 4 weeks since previous chemotherapy, and no coincidental hormonal, immunologic, or radiotherapy.

IL-2 was from Roche Laboratories (Nutley, NJ), as provided by the Biological Response Modifiers Program of the National Cancer Institute, which supported these studies. IL-2 was given i.v. as a bolus over 15 min or continuously over 24 h for 7 consecutive days in doses of 1 x 10^6, 1 x 10^7, 3 x 10^7, and 1 x 10^8 units/ml/day. Three patients were treated at each dose level and by each route of administration, except that only two patients were treated at the 3 x 10^7 continuous level and only two patients received the highest dose of 1 x 10^8 units/ml (one by each route), because of undue toxicity requiring that the treatment be discontinued after 4 days. Complete 24-h urine samples were collected before treatment, daily during the week of treatment, and at 1 and 3 weeks after treatment was stopped. Heparinized blood samples for plasma preparation were taken between 7 and 8 a.m. after overnight fast before treatment, at 2 and 4 h after initiation of treatment, between 7 and 8 a.m. daily thereafter during the week of treatment, at 2 and 4 h after the last dose on day 7, and at 1 and 3 weeks after treatment was stopped. Plasma and urine samples were frozen promptly and stored at −20°C until analyzed.

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3 The abbreviations used are: IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL-2, interleukin 2; KYN, kynurenine; NEO, neopterin; IFN-γ, γ-interferon.
Analytical Methods. Plasma tryptophan was measured by the fluoromeric method of Denkla and Dewey (17) with internal recovery standards of L-tryptophan. In some cases, this method was confirmed by high pressure liquid chromatography assay of tryptophan (18). Urinary KYN was measured by the alkaline steam distillation method, using either manual or autoanalyzer methods developed in this laboratory (19). Urinary NEO was assayed without further oxidation by minor modifications of the high pressure liquid chromatography method of Hausen et al. (20). Routine blood chemistries and patient clinical performance status were monitored.

Statistical Analyses. Changes in tryptophan, KYN, and NEO between pretreatment and posttreatment times were compared by paired t test analyses using arithmetic and logarithmic methods. Comparisons between pretreatment and posttreatment values were made by f tests using a log ratio method. Regression analyses of dose versus metabolite levels were done on data from day 3 (after three doses of IL-2), which was the last day before discontinuance of the highest dose and the time when most consistent changes occurred in individual patients.

RESULTS

In daily values for the parameters studied here, no significant differences were found between bolus and continuous routes of administration of IL-2; therefore, data from both groups of patients were pooled for further study. Fig. 1 shows the changes in plasma tryptophan in the pooled data from subjects receiving the four dosage levels. A dose of 10^5 units/m^2/day did not change tryptophan levels, but higher doses produced a dose-dependent decrease which had not fully returned to pretreatment levels by day 15. Analyses of samples taken 2 and 4 h after the first dose of IL-2 on day 0 and after the last dose on day 6 showed that decreases in plasma tryptophan occurred within 4 h of IL-2 administration, particularly in patients given the 15-min bolus injections. There was a partial restoration of levels in samples taken just before the next daily bolus injection (data not shown). Excretion of the tryptophan catabolite KYN in 24-h urine samples showed dose-related increases corresponding to the decreases in plasma tryptophan (Fig. 2). After cessation of IL-2 administration, KYN levels returned to near pretreatment values with the exception of the group receiving the maximum tolerated dose of 3 × 10^6 units/m^2/day, in which case levels were still significantly elevated on day 15. NEO, a GTP metabolite elevated in a variety of patients having stimulation of the immune system (11-14), was significantly elevated in urine samples following IL-2 administration (Fig. 3).

Dose-response relationships were assessed using data from day 3, since this was the last day that patients receiving 10 × 10^6 units of IL-2 were able to tolerate a full dose. These data are shown in Fig. 4. Significant dose-related changes were observed for plasma tryptophan (P = 0.0001), for urinary KYN (P = 0.0001), and for urinary NEO (P = 0.005). The lines shown in Fig. 4 suggest that there is a sharp response between doses of 10^5 and 10^6 units/m^2/day but that higher doses elicited little further change. However, because of interpatient variability and the limited number of subjects available in this study, this plateau of response could not be established statistically.

In view of the evidence that IFN-γ induces both IDO activity and NEO synthesis in various in vitro systems and that IL-2 can induce IFN-γ production, it seems likely that the metabolic changes observed here were due to the stimulation of IFN-γ production by exogenous IL-2. Consequently, plasma IFN-γ levels were measured in two independent laboratories but none of the samples tested had IFN-γ levels consistently above baseline; however, it remains possible that IFN-γ was produced in vivo at levels with biological activity in the tissues where it was produced but was not detectable in the plasma by the
methods used. Additional evidence that IFN-γ is induced is the observation that urinary NEO levels were also markedly elevated (Figs. 3 and 4). Synthesis of NEO by macrophages is believed to be induced specifically by IFN-γ (10).

These dose-dependent changes in Trp, KYN, and NEO levels correspond to dose-dependent alterations in numerous clinical and immunological parameters observed in these patients and reported elsewhere (21–26). IL-2 could be well tolerated for 4–7 days at doses of 1–3 × 10^6 units/m² by either bolus or continuous infusion (21, 22). A striking rebound lymphocytosis, consisting of lymphocytes bearing activation antigens (la, Leu 17), was observed within 24 h after stopping of IL-2, with a greater rebound observed after continuous infusions than after bolus injections (22). Lymphocytes from this time of lymphocytosis have augmented IL-2 proliferative responsiveness, natural killer cell activity (16), and lymphokine-activated killer cell activity (23–25). Prolonged treatment for 4 weeks with IL-2, using a similar dose and schedule, induced reproducible, measurable antitumor responses in 3 of 23 treated patients (26).

DISCUSSION

The in vivo induction of tryptophan metabolism in humans by IL-2 treatment reported here supports and extends the initial reports (1–3) of induction of IDO activity in mouse tissues by IFN (type 1) or by virus infection, bacterial lipopolysaccharide, or tumor growth in an allogeneic host. Stimulation of NEO excretion in our subjects is also consistent with reports that immune-stimulated patients excrete elevated NEO and that its measurement may be a useful early marker of transplant rejection and other immune-related events (10–15). In the present study with IL-2, changes in urinary KYN are as large or larger than those of NEO and suggest that KYN excretion may also be an early, sensitive, and reliable marker of immune stimulation. The decreased plasma tryptophan observed in the present study is most likely the result of increased tryptophan catabolism via IDO activity, rather than the result of a poor tryptophan intake. In the latter case (27), we observed decreases in urinary KYN rather than increases as found in the present studies.

Earlier studies from this laboratory and others reported disturbances of tryptophan metabolism in a variety of cancer patients (28–30) and in patients with autoimmune diseases such as rheumatoid arthritis, scleroderma, and Wegner’s granulomatosis (31–33). Prior to the discovery of the IDO enzyme, it was believed that certain of these abnormalities of tryptophan metabolism were the result of stress or hormonal induction of liver tryptophan degradation or due to a functional deficiency of vitamin B-6 causing a block in further KYN metabolism (34). With the discovery of IDO and its induction by IFN, and with reports of elevated IFN levels in several autoimmune diseases (35), it now seems most likely that many of the previously reported abnormalities of tryptophan metabolism were the result of IFN-induced IDO activity rather than enhanced activity of the liver enzyme (36).

In view of the essential nature of tryptophan, not only for protein synthesis but also for synthesis of the neuroimmuno-modulator serotonin and the vitamin niacin, it is important to consider the possible physiological and nutritional implications of the marked decrease in plasma tryptophan observed in the present study. Such decreases in tryptophan could readily lead to inhibition of protein synthesis and to low levels of serotonin, potentially causing neurological, vascular, and muscular changes. Additionally, serotonin may be involved in release of pituitary prolactin and growth hormone (37), in regulation of interferon-induced la expression on macrophages (38), and in release or processing of messenger RNA from the cell nucleus (39). Thus, a variety of secondary effects may also result from this IL-2- and IFN-induced decrease in plasma tryptophan. If the toxic or biological effects of systemically administered IL-2 or IFN-γ are, even in part, due to this induced tryptophan deficiency, then the provision of exogenous tryptophan (or a related molecule such as 5-hydroxytryptophan, a serotonin precursor) may potentially ameliorate some of these toxicities. On the other hand, if metabolites of tryptophan and KYN are responsible for toxicity, especially when IDO levels are increased, as suggested by other data (40, 41), then supplementation with tryptophan may enhance toxicity and might not be desirable. Determining the toxic versus protective effects of tryptophan or serotonin in animals receiving high doses of IL-2 or IFN-γ should resolve this issue.

Further testing of IL-2 is required before its clinical utility is defined. Current data suggest its actions may be entirely via its immunopotentiating effects on lymphocytes, particularly a heterogeneous population of effector cells designated lymphokine-activated killer cells which includes subpopulations of T-cells and natural killer cells. The potential clinical benefits of IL-2 in cancer are believed to be based on its induction of antitumor immunological activity. In this setting, the provision of exogenous tryptophan (or congeners) would not be expected to interfere with the desired effect of IL-2, could potentially ameliorate some of the toxic effects, and may enhance tumor cell killing by IFN-γ-activated macrophages (42). However, if serotonin plays a role in regulation of expression of la cell surface markers, as shown in mouse studies (38), then alterations of tryptophan metabolism may also play an indirect regulatory role in the magnitude of the immune-mediated tumor destruction activated by IL-2.

The administration of IL-2 is known to induce secretion of IFN-γ in vitro. High doses of IL-2, particularly by bolus administration, to patients with cancer have caused transient but detectable levels of IFN-γ in the serum (43). It remains uncertain whether the toxicities of IL-2 and the alterations in tryptophan metabolism by IL-2 are, at least in part, due to the direct effect of endogenous IFN-γ induced after IL-2 administration. Patients treated in this study did not have any life-threatening toxicity and the IL-2 at these doses was relatively well tolerated, without any need for intensive care unit monitoring or support. IFN-γ levels were evaluated at multiple times for several of the patients receiving ≥10⁶ units IL-2/m²/day but at no time were significant elevations noted. However, in vitro studies with human lymphocytes show potent induction of IDO by IL-2 which is inhibited by antisera reactive against IFN-γ (44). Thus, it seems most likely that the induction of IDO and NEO production are mediated, at least in part, via induction of IFN-γ by IL-2. The antiproliferative effects of IFN-γ against some human cell lines in culture are apparently mediated by induction of a tryptophan deficiency and are relieved by supplementation of media with tryptophan (45, 46). It, therefore, seems possible that at least some antitumor effects of IL-2 in vivo may be the result of induced IFN-γ, which may act indirectly via depletion of tryptophan.

As IL-2 treatment trials have expanded, it is becoming increasingly important to identify how toxicity and immune activation relate to the potential for the antitumor response in an individual patient. Cellular immune function studies in vitro can be performed reproducibly for single institution trials but are difficult to standardize for large multicenter cooperative trials. In contrast, evaluation of tryptophan and NEO metabo-
lism by analysis of plasma and/or urine is rapid and inexpensive and can easily be performed on cryopreserved plasma and urine specimens tested in a single reference laboratory. Further studies of these metabolic changes are required to determine whether they may quantitatively correlate with immune activation, toxicity, or antitumor responses.

REFERENCES


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