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SHORT COMMUNICATION

Decrease in Hepatic Cytochrome P450 after Interleukin-2 Immunotherapy

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ABSTRACT. Interleukin-2 (IL-2) has been shown to decrease cytochrome P450 (CYP) mRNAs and proteins in cultured rat hepatocytes, and IL-2 administration decreases CYPs in rats. Although high doses of IL-2 are administered to cancer patients, the effect on human CYPs has not yet been determined. Patients with hepatic metastases from colon or rectum carcinomas were randomly allocated to various daily doses of human recombinant IL-2 (from 0 to 12.10^6 units/m^2). IL-2 was infused from day 7 to day 3 before hepatectomy and the conservation of a non-tumorous liver fragment in liquid nitrogen. Hepatic CYPs and monooxygenase activities were not significantly decreased in 5 patients receiving daily doses of 3 or $6 \cdot 10^6$ IL-2 units/m^2, compared to 7 patients who did not receive IL-2. In contrast, in 6 patients receiving daily doses of 9 or $12 \cdot 10^6$ IL-2 units/m^2, the mean values for immunoreactive CYP1A2, CYP2C, CYP2E1, and CYP3A4 were 37, 45, 60 and 39%, respectively, of those in controls; total CYP was significantly decreased by 34%, methoxyresorufin *O*-demethylation by 62%, and erythromycin *N*-demethylation by 50%. These observations suggest that high doses of IL-2 may decrease total CYP and monooxygenase activities in man. BIOCHEM PHARMACOL **57**;8:951–954, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. interleukin-2; immunotherapy; cancer; cytochrome P450; liver; drug metabolism

IL-2¶ plays a major role in triggering immunological responses [1]. When helper T lymphocytes recognize a foreign peptide on antigen-presenting cells, the expression of IL-2 and IL-2R is induced in the helper T cells [1]. The binding of IL-2 to the IL-2R mediates the T cell proliferation and differentiation signal [2]. This signal first activates tyrosine kinases [2], which can be blocked with genistein, a tyrosine kinase inhibitor [3]. The IL-2/IL-2R interaction also activates natural killer lymphocytes that can lyse tumor cells [4, 5]. Despite the poor clinical tolerance of these treatments, IL-2 is used in humans to treat some advanced cancers [6–8]. Due to the rapid renal clearance of IL-2, high doses are required: $45 \cdot 10^6$ IL-2 units daily when three successive intravenous injections are administered [7] or 9 or $12 \cdot 10^6$ IL-2 units/m^2 daily for continuous infusion [8].

We recently showed that the IL-2R is expressed on the surface of rat hepatocytes and that IL-2 down-regulates the expression of CYPs in primary cultures of rat hepatocytes [9]; this effect is blocked by either a monoclonal antibody against the IL-2R or by genistein, indicating that it is mediated by the IL-2R [9]. Although the administration of human recombinant IL-2 decreases the expression of CYPs in rats [10], the effect of IL-2 treatments on CYP expression in humans has not yet been determined.

Thus, an exploratory study of hepatic CYPs was performed in 18 patients with or without IL-2 treatment before surgical resection of hepatic metastases and the conservation of a non-tumorous liver fragment in liquid nitrogen.

MATERIALS AND METHODS

Patients

The study was part of a phase I–II randomized study of the tolerance and immunological effects of human recombinant IL-2; results on the tolerance and immunological effects of IL-2 have been reported [11]. This study was approved by the Trial Evaluation Committee of the Institut Gustave Roussy and the Regional Ethics Committee. Patients were fully informed before providing written consent. The study was performed in patients with surgically resectable hepatic metastases from adenocarcinomas of the colon or rectum [11]. Patients were randomly assigned to the control group

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¶ *Abbreviations:* IL, interleukin; IL-2R, interleukin-2 receptor; and CYP, cytochrome P450.

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TABLE 1. Effects of IL-2 on CYP isoenzymes, monooxygenase activities, and total CYP*

Daily IL-2 units (10^6/m^2)	No. of subjects	CYP isoenzymes (% of control)				Monooxygenase activities (pmol or nmol/min/mg protein)		Total CYP (nmol/mg protein)
		CYP1A2	CYP2C	CYP2E1	CYP3A	Methoxyresorufin	Erythromycin	
0	7	100 ± 37	100 ± 43	100 ± 27	100 ± 43	1.6 ± 0.6	1.75 ± 0.65	0.50 ± 0.06
3 or 6	5	101 ± 42	91 ± 30	59 ± 16	69 ± 21	1.3 ± 0.6	1.82 ± 0.81	0.46 ± 0.03
9 or 12	6	37 ± 17	45 ± 22	60 ± 21	39 ± 6	0.6 ± 0.2†‡	0.88 ± 0.36†‡	0.33 ± 0.04†

Microsomes were prepared from the non-tumorous liver. CYP isoenzymes were quantitated by laser densitometry of immunoblots. Methoxyresorufin (2.5 μM) *O*-demethylation (pmol/min/mg) was assayed by measuring the formation of the fluorescent resorufin. Erythromycin (1 mM) *N*-demethylation (nmol/min/mg) was assayed by measuring the formed formaldehyde with the Nash reagent. Total CYP was measured from the CO-difference spectrum of dithionite-reduced microsomes; because not enough microsomes remained in four patients, total CYP was determined in 5, 4, and 5 patients, respectively. All results are means ± SEM.

*Patients with hepatic metastases from colorectal cancers were treated with IL-2 (0, 3, 6, 9, or 12 · 10^6 units/m^2) daily from day 7 to day 3 before hepatectomy.

†Different from untreated patients, Dunnett's *t*-test and/or Fisher's test ($P < 0.05$).

‡Different from untreated patients, non-parametric Mann and Whitney test ($P < 0.05$).

or to various daily doses of IL-2 (either 3, 6, 9, or 12 · 10^6 units/m^2) [11]. IL-2 was continuously infused with a perfusion pump from day 7 to day 3 before surgery. The protocol allowed administration of three other drugs for the symptomatic treatment of IL-2-induced fever, nausea, or diarrhea during the period of IL-2 administration [11]. Paracetamol (2 g every 6 hours) was systematically administered to all IL-2-treated patients. Metoclopramide (300–400 mg daily) and loperamide (6 mg daily) were administered only in cases of nausea or diarrhea, respectively; these two drugs were used in four IL-2-treated patients (2 patients receiving doses of 9 IL-2 units/m^2 and 2 individuals treated with 12 IL-2 units/m^2). Like IL-2, these three drugs were not administered on the 2 days preceding surgery. The tumorous liver was removed by various types of hepatectomy, and a non-tumorous liver fragment was frozen in liquid nitrogen. A frozen fragment remained available for CYP isoenzyme measurements in 18 patients, including 7 patients who had not been treated with IL-2, 3 patients treated with 3 · 10^6 IL-2 units/m^2 daily, 2 patients treated with 6 · 10^6 units/m^2 daily, 2 patients with 9 · 10^6 units/m^2 daily, and 4 patients with 12 · 10^6 units/m^2 daily.

Anti-CYP Antibodies, Total CYP, CYP Proteins, and Monooxygenase Activities

The nomenclature of Nelson *et al.* [12] is used in this report. The characteristics of human liver CYP1A2, CYP2C, CYP2E1, and CYP3A4 and their antibodies have been described [13–18]; polyclonal antibodies against human CYPs1A, CYPs2C, and CYP2E1 were raised in rabbits; the preparation of a mouse monoclonal antibody against human CYPs3A has been described [17].

Microsomes were isolated by centrifugation at 10,000 and then 100,000 *g*, and were resuspended in 20% glycerol, 1 mM EDTA, 0.1 M phosphate buffer, pH 7.4 [19]. The microsomal suspension was stored at −80° until use. Ten μg of microsomal proteins were fractionated by sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis as described [19]. Electrophoretic transfer to nitrocellulose sheets was performed in a Bio-Rad semidry transfer cell (Biorad). Unspecific binding sites were blocked with 1% Tween 20 and 1% polyvinylpyrrolidone in PBS, pH 7.2. Nitrocellulose sheets were first incubated with the anti-CYP antibody diluted 1:1000 in PBS containing 10% bovine serum albumin and 10% neonatal bovine serum. Sheets were then incubated with a peroxidase-conjugated antibody against rabbit or mouse immunoglobulins (DAKO), and stained with diaminobenzidine and H_2O_2 in the presence of 0.015% $CoCl_2$ and 0.015% $NiCl_2$ [19]. Total CYP was measured by the CO-difference spectrum of dithionite-reduced microsomes [19]. Methoxyresorufin (2.5 μM) *O*-demethylation, an activity supported by CYP1A2, and erythromycin (1 mM) *N*-demethylation, an activity supported by CYPs 3A, were measured as previously reported [10].

Statistical Analysis

Data in control subjects, subjects treated with 3–6 · 10^6 IL-2 units/m^2, and subjects treated with 9–12 · 10^6 IL-2 units/m^2 were first compared by ANOVA followed by a Dunnett's *t*-test and a Fisher's test. Differences between control subjects and subjects treated with 9–12 · 10^6 IL-2 units/m^2 were also assessed by the non-parametric test of Mann and Whitney.

RESULTS

Previously reported immunologic studies indicated that daily doses of either 3 or 6 · 10^6 IL-2 units had limited immunologic effects, whereas daily doses of either 9 or 12 · 10^6 IL-2 units markedly increased blood lymphocytes and their natural killer activity in these patients [11]. Similarly, in the present study, CYP results showed little or no change in patients receiving the two lowest daily doses of IL-2, but more marked changes with the two highest doses. Therefore, IL-2-treated patients were subdivided into 2 groups: patients treated with either 3 or 6 · 10^6 IL-2 units/m^2 daily and patients treated with either 9 or 12 · 10^6 IL-2 units/m^2 daily (Table 1).

CYP proteins, monooxygenase activities, and total CYP were not significantly decreased in the group of patients

treated with 3 or $6 \cdot 10^6$ IL-2 units/m^2 (Table 1). In patients receiving daily doses of 9 or $12 \cdot 10^6$ IL-2 units/m^2, the mean values for immunoreactive CYP1A2, CYP2C, CYP2E1, and CYP3A4 were 37, 45, 60, and 39%, respectively, of those in controls (Table 1). These differences were not statistically significant, probably due to the high interindividual variability of CYP isoenzymes and the small number of patients (Table 1). However, total CYP was significantly decreased by 32%, methoxyresorufin *O*-demethylation by 63%, and erythromycin *N*-demethylation by 50% (Table 1).

DISCUSSION

Three other drugs were administered during the period of IL-2 administration, and were stopped, like IL-2, on the two days preceding surgery. Metoclopramide and loperamide were administered to 4 IL-2-treated patients. Metoclopramide is mainly eliminated in urine as the unchanged drug and its sulfate and glucuronic acid conjugates [20]. Loperamide is also eliminated as the unchanged drug, but a fraction is demethylated [21], presumably by CYP. Neither of these *in vivo* treatments is known to inhibit, destroy, or down-regulate CYPs [20–23]. Paracetamol was administered to all IL-2-treated patients, whatever the IL-2 dose. In contrast, CYP effects were clearly dependent on the dose of IL-2. No significant CYP change occurred at daily doses of 3 or $6 \cdot 10^6$ IL-2 units/m^2, whereas significant decreases in total CYP and monooxygenase activities were observed at daily doses of 9 or $12 \cdot 10^6$ IL-2 units/m^2 (Table 1). Thus, it can be reasonably assumed that CYP decreases were caused by IL-2 administration.

Patients underwent surgery two days after the IL-2 infusion was stopped. Exogenous IL-2 was probably cleared at the time of surgery. However, high IL-2 doses caused marked lymphocyte stimulation [11]. On the day of surgery, blood lymphocytes were increased 2-fold both in patients receiving 3 and in those receiving $6 \cdot 10^6$ IL-2 units/m^2 daily [11]. Blood lymphocytes were increased 5-fold both in patients receiving 9 and in those receiving $12 \cdot 10^6$ IL-2 units/m^2 [11]. The cytotoxicity of blood lymphocytes against K562 and DAUDI tumor cell lines was barely increased after administration of 3 or $6 \cdot 10^6$ IL-2 units/m^2, but markedly increased after 9 or $12 \cdot 10^6$ IL-2 units/m^2 [11]. Activated lymphocytes secrete endogenous IL-2 and various other cytokines [1]. The IL-2/IL-2R interaction has been shown to down-regulate the expression of CYP mRNAs and CYP proteins in cultured rat hepatocytes [9], and several other cytokines, including IL-1, IL-6, tumor necrosis factor-α, and interferons also repress CYP transcription in rat or human hepatocytes [24, 25]. Thus, the effects of high doses of IL-2 on CYPs (Table 1) may be due to the combined effects of exogenous IL-2, endogenous IL-2, and other endogenous cytokines.

Interferon is also used therapeutically in humans, and has been shown to decrease hepatic drug metabolism and cause drug interactions in man [26]. The present study suggests that the IL-2 doses ($9–12 \cdot 10^6$ units daily) that are currently employed in cancer patients [8] may likewise cause drug interactions.

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