Changes in Laboratory Results for Cancer Patients Treated with Interleukin-2
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The systemic administration of interleukin-2 (IL-2) can lead to significant antitumor responses in some patients with metastatic cancer in whom standard therapy has failed. A limitation of this immunotherapy is the toxicity associated with IL-2 infusion. To assess toxicity, we determined aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2), gamma-glutamyltransferase (GGT; EC 2.3.2.2), lactate dehydrogenase (LD; EC 1.1.1.27), alkaline phosphatase (ALP; EC 3.1.3.1), creatine kinase (CK; EC 2.7.3.2), total bilirubin (TBI), direct bilirubin (DBI), creatinine, urea nitrogen, and C-reactive protein in serum from 21 patients before and during five consecutive days of IL-2 treatment. Ten patients were followed for an additional five days after the end of IL-2 therapy. The IL-2 infusion caused liver toxicity and prerenal azotemia, as evidenced by significant increases (P < 0.05) of all analytes except CK by day 1. There was a progressive increase in the results (except CK) for these tests until IL-2 treatment was stopped. Seven tests related to liver function (AST, ALT, GGT, LD, ALP, DBI, and TBI) showed increases, but the test results indicated significant improvement and moved toward the baseline value five days after the end of IL-2 therapy. Concentrations of creatinine and urea nitrogen in serum were normal three days after the cessation of IL-2 therapy.

Additional Keyphrases: liver and kidney toxicity • chemotherapy

Interleukin-2 (IL-2), previously called T-cell growth factor, is a 15,000-Da glycoprotein that is released by a subpopulation of activated T lymphocytes during an immune response (1). A potent lymphokine, IL-2 mediates several immunoregulatory events, both in vitro and in vivo. In vivo, IL-2 enhances the activity of natural killer cells, increases alloantigen responses, and improves the recovery of immune function in immunocompromised patients (1). Perhaps the most important role of IL-2 is to directly induce the production of several other lymphokines, including the tumor necrosis factor (cachectin, TNF), gamma-interferon (2–6), and interleukin-6 (IL-6) (7).

Some patients with metastatic melanoma, renal-cell carcinoma, or colon cancer, in whom standard therapy has failed, have responded to treatment with IL-2 alone (8, 9) or in conjunction with the infusion of autologous lymphokine (IL-2)-activated killer (LAK) cells (9–11). A limitation of this immunotherapy is the toxicity associated with IL-2 administration. The major side effects include those secondary to the vascular leak syndrome, which is associated with fluid retention, pulmonary interstitial edema, respiratory distress, and hypotension (9–12). In addition, transient fever and chills, general malaise, nausea, vomiting, diarrhea, hyperbilirubinemia, azotemia, and oliguria are observed with IL-2 treatment. Here we assess changes in laboratory test results for liver and renal toxicities during and immediately after IL-2 therapy.

Materials and Methods

Patient population: We studied 21 patients with metastatic cancer, in whom standard therapy had failed. Twelve patients had malignant melanoma, six had renal-cell carcinoma, and three had colon cancer. Signed informed consent was obtained from all of the patients before entry into this Investigational Review Board-approved trial. Most of the patients received five daily of IL-2 therapy (106 units/kg of body weight, intravenously, three times daily). Eleven patients continued to receive immunotherapy with LAK cells and were not analyzed for the second part of the study. Patients were monitored for clinical symptoms of myocardial infarction.

Interleukin-2: Recombinant IL-2 was produced in Escherichia coli cells transfected with the gene for IL-2, isolated from the Jurkat cell line. The recombinant IL-2 used in this trial was kindly supplied by Cetus Corporation (Emeryville, CA), which had purified the IL-2 to homogeneity and extensively studied its biological characteristics. A lyophilized powder of IL-2 was reconstituted with 1.2 mL of sterile water per vial. Each vial contained 0.3 mg of IL-2 (specific activity 3 × 106 to 5 × 106 units per milligram).

Administration of recombinant IL-2: Recombinant IL-2 was diluted in 50 mL of isotonic saline (NaCl 8.5 g/L) containing human serum albumin, 50 g/L, and was infused intravenously over a 15-min period every 8 h for five days. Although doses were occasionally omitted, depending on the patient’s tolerance, each patient received a cumulative dose of IL-2 of approximately 100,000 units/kg over the five-day period. The protocol for the administration of IL-2 has been published (13).

Biochemical analysis: We used a smac continuous-flow analyzer (Technicon Instruments Corp., Tarrytown, NY 10591) to determine the activities of AST, ALT, ALP, LD, and CK, and to quantify the concentrations of TBI, DBI, BUN, and creatinine. An aca clinical analyzer (Du Pont Co., Wilmington, DE) was used to determine the activities of GGT. We used an immunochromatography system (ICS; Beckman Instruments Inc., Fullerton, CA) to determine CRP concentration.

Statistical analysis: Results are expressed as the mean ± SEM. We used the Wilcoxon signed-rank test (14) to assess
the significance of the difference from baseline concentration (day 0) with results from days after IL-2 administration.

Results

Liver function: Figure 1 and Table 1 show the changes of ALT, AST, GGT, ALP, LD, TBI, DBI, and DBI/TBI after the initiation of IL-2 treatment. For most patients, results for liver-function tests were within the reference interval before treatment with IL-2 (Table 1). The mean ± SEM baseline values were as follows: ALT, 27.1 ± 5.5 U/L; AST, 20.1 ± 2.2 U/L; GGT, 38.4 ± 11.5 U/L; ALP, 89.2 ± 6.7 U/L; LD, 154 ± 7.6 U/L; TBI, 6.0 ± 0.6 mg/L (10.3 ± 0.86 μmol/L); and DBI, 0.9 ± 0.1 mg/L (1.54 ± 0.17 μmol/L). The IL-2 infusion caused immediate changes in liver-function results as evidenced by significant increases (P < 0.05) for these seven analytes and the DBI/TBI ratio at day 1 of IL-2 treatment compared with day 0. Liver enzymes, TBI, DBI, and DBI/TBI continued to increase over the five days of IL-2 treatment. The peak concentration for all seven analytes occurred close to the end of IL-2 treatment (day 0 vs day 5; P < 0.0005, Table 1). The results for the seven liver-function tests progressed toward the baseline value after discontinuation of IL-2 infusion. However, all analytes were still significantly greater at day 10 than at day 0 (Table 1).

Renal function: Serum creatinine and urea nitrogen concentrations before IL-2 treatment were 11.3 ± 0.07 mg/L (99.9 ± 0.6 μmol/L) and 151 ± 10 mg/L (2.51 ± 0.17 mmol/L), respectively (Table 1). Both increased significantly one day after IL-2 administration (P < 0.05), continued to increase thereafter, and reached a plateau at about day 5 at about double the baseline concentration (P < 0.005; Figure 1 and Table 1). The urea nitrogen/creatinine ratio increased by a factor of 1.8 after five days of IL-2 therapy. The creatinine and urea nitrogen concentrations from day 8 through day 10 were not significantly different from the baseline concentrations (P > 0.05).

Fig. 1. Changes in laboratory tests during and after IL-2 therapy
Results are expressed as the difference between the result during and after IL-2 therapy and the baseline value (time 0). Values for individual patients are shown, and the mean (±1 SEM) values for each day are connected by the solid line. IL-2 was administered to the patients during the first five days of the study.
Table 1. Changes in Laboratory Results (Mean ± SEM) during and after IL-2 Therapy of Cancer Patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Day 0 value</th>
<th>Peak day*</th>
<th>Peak day values/day 0 values</th>
<th>Day 10 values/day 0 values</th>
<th>( P^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT, U/L</td>
<td>27.1 ± 5.5</td>
<td>4.8 ± 0.6</td>
<td>6.2 ± 1.0 (21)(^c)</td>
<td>2.9 ± 0.5 (10)</td>
<td>0.003</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>20.1 ± 2.2</td>
<td>4.3 ± 0.6</td>
<td>6.1 ± 0.9 (21)(^c)</td>
<td>2.3 ± 0.5 (10)</td>
<td>0.014</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>89.2 ± 6.7</td>
<td>5.6 ± 0.2</td>
<td>4.8 ± 0.6 (21)(^c)</td>
<td>2.2 ± 0.3 (10)</td>
<td>0.003</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>38.4 ± 11.5</td>
<td>6.1 ± 0.4</td>
<td>11.5 ± 1.7 (17)(^d)</td>
<td>6.8 ± 1.8 (6)</td>
<td>0.016</td>
</tr>
<tr>
<td>LD, L/U</td>
<td>154 ± 7.6</td>
<td>5.0 ± 0.5</td>
<td>1.8 ± 0.1 (21)(^c)</td>
<td>1.6 ± 0.1 (10)</td>
<td>0.001</td>
</tr>
<tr>
<td>TBI, mg/L</td>
<td>6.0 ± 0.6</td>
<td>5.2 ± 0.2</td>
<td>15.4 ± 3.1 (21)(^d)</td>
<td>3.3 ± 0.7 (10)</td>
<td>0.002</td>
</tr>
<tr>
<td>DBI, mg/L</td>
<td>0.9 ± 0.1</td>
<td>5.8 ± 0.5</td>
<td>52.1 ± 8.4 (14)(^d)</td>
<td>12.6 ± 2.3 (7)(^d)</td>
<td>0.008</td>
</tr>
<tr>
<td>DBI/TBI</td>
<td>0.12 ± 0.02</td>
<td>5.5 ± 0.5</td>
<td>5.3 ± 0.4 (14)(^d)</td>
<td>3.0 ± 0.4 (7)(^d)</td>
<td>0.008</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>27.5 ± 7.0</td>
<td>4.0 ± 0.6</td>
<td>7.7 ± 1.7 (15)(^d)</td>
<td>0.6 ± 0.3 (5)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>CK, U/L</td>
<td>57.4 ± 6.3</td>
<td>2.0 ± 0.5</td>
<td>2.4 ± 0.5 (21)(^d)</td>
<td>0.4 ± 0.1 (10)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>CRE, mg/L</td>
<td>11.3 ± 0.7</td>
<td>4.3 ± 0.1</td>
<td>2.0 ± 0.1 (21)(^d)</td>
<td>1.1 ± 0.05 (10)</td>
<td>0.16</td>
</tr>
<tr>
<td>BUN, mg/L</td>
<td>151 ± 10</td>
<td>5.4 ± 0.8</td>
<td>2.4 ± 0.3 (16)(^d)</td>
<td>0.8 ± 0.1 (10)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>BUN/CRE</td>
<td>13.4 ± 1.4</td>
<td>5.1 ± 0.8</td>
<td>1.8 ± 0.7 (16)(^d)</td>
<td>0.7 ± 0.1 (7)</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>

* Time in days after start of treatment.
\( P \) P value for day 10/day 0 determined with the Wilcoxon signed-rank test.
\( c \) The number in parentheses represents the number of patients.
\( d \) Specimens with direct bilirubin = 0 eliminated.

Acute-phase protein: CRP showed a significant increase at day 1 \(( P < 0.005)\) of treatment. The peak increase (7.8 times the baseline) occurred at day 3. However, one day after discontinuation of IL-2 (day 6) and thereafter, the CRP concentration did not differ significantly from the baseline value (Figure 1).

Creatine kinase: The results for creatine kinase differed from the other enzymes: it peaked earlier (day 2) and showed no significant increases from the baseline activity during the study.

Discussion

IL-2-based immunotherapy has proven useful in some patients with advanced cancer in whom standard therapy has failed. The major obstacle of this regimen is the toxicity, associated primarily with IL-2 administration. Our previous observations had suggested that most of the toxicity was attributable to the vascular leak syndrome, which was associated with substantial fluid retention and respiratory distress \((10)\). The systemic IL-2 infusion caused immediate liver toxicity and prerenal azotemia, as shown by a significant increase \(( P < 0.05)\) in the results of the biochemical tests for these functions. The abnormalities of these functions showed progressive increases, peaking near the end of IL-2 therapy \((day 5)\). These data suggest that the toxicity is related to the cumulative dose of IL-2 over consecutive days of therapy.

The nature of the hepatic dysfunction is not entirely clear and may have several causes. The increase in amino-transferases, alkaline phosphatase, and total bilirubin, of which about 75% was direct bilirubin, was consistent with intrahepatic cholestasis. \(^{31}P\)c-Disofenin scans performed in patients receiving IL-2 treatments showed normal uptake of tracer by the liver but a markedly delayed excretion into the biliary tree, gall bladder, and small bowel \((15)\)—also findings consistent with cholestasis. Increases of GGT and ALP, membrane-bound cholestasis markers, are presumably caused by the increased growth of hepatic and bile duct epithelium and a concurrent increase of cell permeability \((16)\). Increased liver enzyme activities observed in the patients may be in part induced by IL-6 (hepatocyte-stimulating factor) or other induced cytokines acting on the liver. IL-2 has recently been shown to increase the concentration of both TNF and IL-6, which in turn cause increases of enzyme activities traditionally used for liver-function tests (Huang et al., unpublished results). The magnitude of liver parenchymal damage may not be as severe as the activities of these liver enzymes indicate. Liver enzyme activities varied markedly in the patients receiving IL-2 infusion. We previously observed variable production of TNF and IL-6 \((7)\), which may be a factor for the variability in increases of enzyme activities.

The serum creatinine and urea nitrogen concentrations were significantly increased during IL-2 infusion and for two days afterwards. A previous study showed no evidence of renal tubular dysfunction with IL-2 therapy \((17)\); thus, the increase in the serum creatinine and urea nitrogen concentrations is probably due to prerenal azotemia. This would be consistent with the clinical syndrome of hypotension, oliguria, and azotemia and the increase in the ratio of urea nitrogen to creatinine during IL-2 therapy. The increased creatinine and urea nitrogen concentrations may be due to decreased perfusion of the kidney and a decrease in renal prostaglandin synthesis at a time of increased plasma renin activity in patients receiving IL-2 therapy \((18)\). Fortunately, the prerenal azotemia induced by IL-2 infusion was reversible. The creatinine and urea nitrogen concentrations in serum returned to the baseline three days after the cessation of IL-2 therapy.

CRP was significantly increased one day after IL-2 therapy was begun. The acute-phase response and the vascular leak syndrome induced by IL-2 effect increased CRP synthesis by hepatocytes. The vascular leak syndrome was often observed within 24 h of an IL-2 infusion, with clinical features of extracellular fluid accumulation including ascites, hydrothorax, and pulmonary edema. The precise pathogenesis of this toxic effect is unclear. We suspect widespread inappropriate endothelial cell activation, as evidenced by the detection of three different activation antigens \((12)\). Not only do products of activated cells have the capacity to initiate an acute-phase response and thereby increase CRP concentration \((19)\), but also IL-6 has been shown to be a major and direct inducer of CRP synthesis in several different human hepatoma cell lines \((20)\). Jablons et al. \((7)\) also observed a significant increase in CRP concentration in patients receiving high doses of...
recombinant TNF, which stimulated IL-6 production (7). CRP is synthesized mainly in hepatocytes, the rate of synthesis being directly related to the duration and the extent of the inflammation in vivo. In view of the short half-life of CRP (approximately 8.9 h), the post-inflammatory elimination of CRP is equally rapid (19), and probably explains why the CRP concentration did not differ significantly from the baseline value one day after cessation of IL-2 therapy.

Clinical and laboratory information indicates that none of the patients had a myocardial infarction during the study. No clinical symptoms of myocardial infarction were reported for any of the patients, and mean CK activity did not increase significantly from the baseline. Thus, myocardial infarction was not a factor affecting our results.

References