J Cancer Res Clin Oncol (1989) 115:175–178

Journal of Cancer Research and Clinical Oncology

Severe intrahepatic cholestasis in patients treated with recombinant interleukin-2 and lymphokine-activated killer cells*

Mitchel Hoffman, Abraham Mittelman, Brad Dworkin, William Rosenthal, Deborah Beneck, Elizabeth Gafney, Zalmen Arlin, Daniel Levitt, and Eckhard Podack**

Departments of Medicine, Immunology and Pathology, New York Medical College, Vahalla, NY 10595, USA
** Hoffmann-La Roche Inc., Nutley, NJ 07110, USA

Summary. Immunotherapy with recombinant interleukin-2 (rIL-2) and lymphokine-activated killer cells (LAK) has become a new form of therapy that has been shown to induce remissions in patients with renal cell carcinoma and melanoma. Despite encouraging results, this form of therapy has been associated with increasing toxicity, often requiring therapy in an intensive-care unit. In this report severe intrahepatic cholestasis occurred in two patients receiving rIL-2 and LAK cells. This form of cholestasis appeared to be directly related to rIL-2 administration at a doses of 2×10^6 U/m^2 and 3×10^6 U/m^2 t.i.d. A liver biopsy showed moderate hepatocellular bile stasis, with lobular and portal inflamation. All other studies for potential cause of this cholestasis were negative, including studies for metastatic disease. When therapy was discontinued, evidence for cholestasis and bile stasis resolved. We conclude that rIL-2 is a drug with a potential to induce severe hepatic injury that is reversible upon cessation of therapy with rIL-2. Further care should be exercised when rIL-2 is administered to patients with abnormal liver function.

Key words: Interleukin-2 – LAK cells – Cholestasis

Introduction

Adoptive immunotherpy with recombinant interleukin-2 (rIL-2) and lymphokine-activated killer cells (LAK) has recently been shown to induce remissions in selected patients with metastatic cancers (Rosenberg et al. 1985, 1987; West et al. 1987; Lotze et al. 1986; Hsieh et al. 1987). These preliminary encouraging results, however, have been associated with severe toxic reactions, often requiring therapy in intensive-care units. The major toxic effects of rIL-2 and LAK included fever, chills, anorexia, fluid retention, hypotension, renal toxicity, and hematological abnormalities such as thrombocytopenia, eosinophilia, and anemia (Rosenberg et al. 1985, 1987; West et al. 1987; Lotze et al. 1984–1986; Atkins et al. 1986). Mild to moderate jaundice has also been previously reported (Rosenberg et al. 1985, 1987; West et al. 1987; Lotze et al. 1984–1986). We now present two cases of severe intrahepatic cholestasis attributed to rIL-2 and LAK in two patients with malignant melanoma.

* Supported in part by Dr. I. Fund Foundation, Hoffmann-La Roche Inc., Nutley, New Jersey, and the Sarah C. Upham Trust
Offprint requests to: A. Mittelman

Case I

In 1981, a 49-year-old man was found to have malignant melanoma on his back and this was removed by surgical wide excision. A year later, he developed recurrence in his right axillary nodes. He received immunotherapy consisting of thymosin fraction 1 weekly for 2 years, with no evidence of recurrence.

In September 1986, he underwent thoracotomy for two nodules in his left lung. Histological evaluation revealed recurrent melanoma. Physical examination 3 months later also revealed multiple nodules in his right testicle, which were consistent with metastatic disease. He was referred for further therapy with rIL-2 and LAK cells. Upon admission, he had a normal complete blood count, prothrombin and partial thromboplastin time. The serum aspartate aminotransferase (AST) was 34 IU/l, serum alanine aminotransferase (ALT) was 35 IU/l; total bilirubin was 0.6 mg/dl; alkaline phosphatase was 69 IU/l; serum γ-glutamyl transpeptidase (GGT) was 138 IU/l. There was no history of alcohol usage, no hepatotoxic drug use, or exposure to hepatitis.

Immunotherapy with rIL-2 was initiated. The treatment schedule consisted of 3.1×10^6 units of rIL-2 intravenously t.i.d. on day 1, leukophoresis on days 3 and 4, and rIL-2 administration again on day 5–18. LAK infusion with continued rIL-2 infusion was started on treatment day 8 for a total of 9 days: a total of 18 days of therapy. An average of 3.8×10^9 cells were infused per day. This treatment schedule was different from the previously described schema in that prolonged LAK cell infusion was carried out: in this trial 9 days in other trials 5 days only.

As shown in Fig. 1, the patient developed cholestasis during the course of therapy with rIL-2 and LAK cells. On day 19 the bilirubin peaked at 21.3 mg/dl. The alkaline phosphatase was 385 IU/l with a normal ALT and AST. The white blood cell count was 19 800 with an

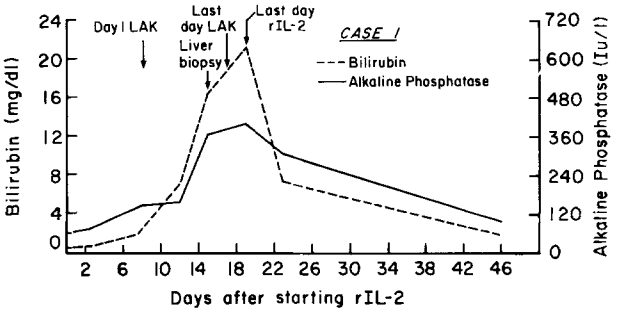

Fig. 1. The development of hyperbilirubinemia and elevated alkaline phosphatase related to recombinant interleukin-2 (rIL-2) and lymphokine-activated killer cells (LAK) adoptive immunotherapy. Complete recovery by day 46 (case I)

absolute count of 3762 eosinophils. The patient developed anorexia, nausea and diffuse myalgias, with no evidence of abdominal pain, rash or pruritus.

On physical examination, the patient was found to have severe icterus and a non-tender liver of normal size. There was no splenomegaly, ascites, encephalopathy or cutaneous manifestations of liver disease. The rest of his physical examination was remarkable only for testicular nodules previously described.

During the hospital course the patient experienced febrile episodes to 40.5 °C and chills that appeared temporally related to rIL-2 infusion. All blood and urine cultures were negative. Additional problems observed in this patient were fluid retention, hypotension requiring dopamine, and anemia necessitating blood transfusion on days 5 and 16. The patient had received medications, including standard doses of prochlorperazine and acetominophen. All patients entered into this trial received the same medication (acetoaminophen and prochlorperazine) throughout this treatment.

IgG (but not IgM) antibody to hepatitis A was present. Hepatitis B surface antigen or antibody to surface or core antigen was not detected. A computerized tomography (CT) scan of the abdomen showed a normal liver and biliary tract.

A liver biopsy was performed on day 15 of therapy and it demonstrated moderate hepatocellular bile stasis without canalicular plugging. Small groups of polymorphonuclear leukocytes were scattered throughout both portal triads and within the lobules, but inflammation was not a predominant feature. Occasional individual necrotic hepatocytes and mitotic figures were present. There was no evidence of metastatic malignant melanoma or of fibrosis or damage to bile ducts (Fig. 2, 3)

When therapy was completed, jaundice began to resolve and 4 weeks later liver function returned to pretherapy levels (Fig. 3).

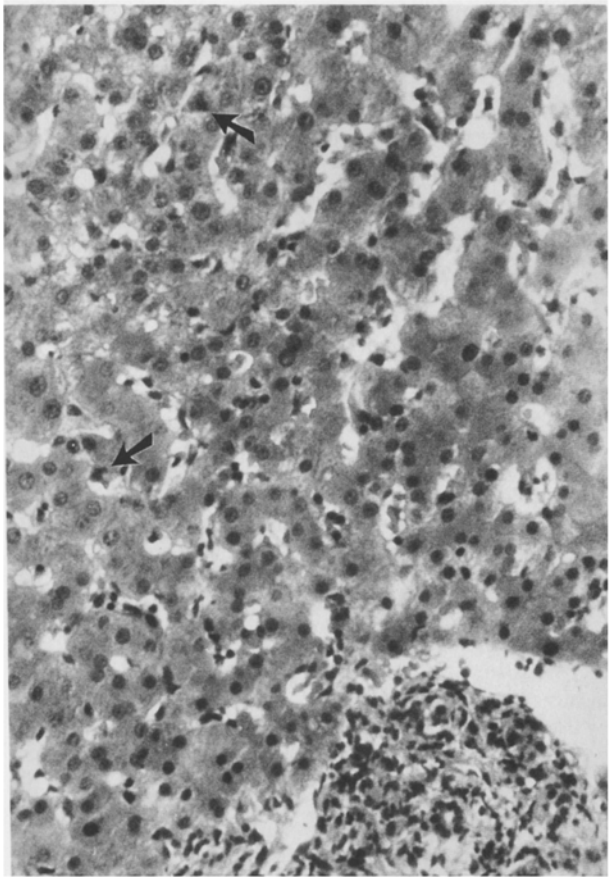

Fig. 2. Liver biopsy, in case I, dense acute and chronic inflammatory infiltrate in portal area with focal extension into lobule and occasional necrotic hepatocytes (*arrows*). (Hematoxylin and eosin; original magnification 100×)

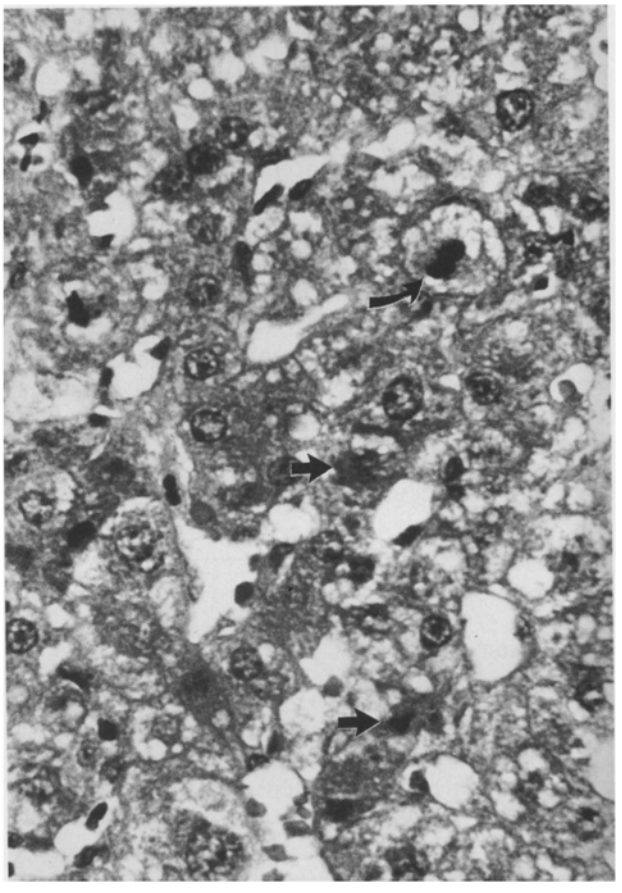

Fig. 3. Liver biopsy, case I. Scattered necrotic hepatocytes (*straight arrows*). Occasional mitotic figures (*curved arrow*) also seen. (Hematoxylin and eosin; original magnification 400×)

Case II

In 1973, a 37-year-old woman was found to have melanoma of her upper extremity and underwent a wide excision. In June 1986, subcutaneous lesions were found and chest and abdominal CT scans revealed the presence of multiple metastatic nodules in the liver and lungs. Two courses of ehcmotherapy were administered which consisted of cisplatinum in combination with interferon α. No evidence of any clinical response was shown, and the patient was referred for further therapy with rIL-2 and LAK.

Upon admission, a complete blood count, prothrombin, and partial thromboplastin time were normal. Liver function tests showed an alkaline phosphatase of 262 IU/l, AST of 29 IU/l, ALT of 28 IU/l, GGT of 140 IU/l, total bilirubin of 0.3 mg/dl and serum albumin of 4.5 g/dl. Therapy with rIL-2 was administered on a schedule described in the previous case at a starting dose of 3×10^6 U/m^2 intravenously t.i.d. As shown in Fig. 4, cholestasis developed approximately 2 weeks after starting therapy. On day 22, the bilirubin peaked at 21.6 mg/dl, and the prothrombin time was 15.9 s with a control of 12.2 s. During the course of therapy, the patient developed a leukocytosis having a white blood cell count of 41 700 with an absolute eosinophil count of 23 732 and a platelet count of 58 000. The jaundice resolved 21 days following cessation of therapy.

After 7 weeks, she was readmitted for a second course of rIL-2 and LAK. Admission liver function tests showed an AST of 57 IU/l, ALT of 75 IU/l, GGT of 186 IU/l, alkaline phosphatase of 384 IU/l and a total bilirubin of 0.8 mg/dl. Therapy with rIL-2 was reinstituted and again the patient developed cholestasis. On day 18 the total bilirubin was 13.9 mg/dl, AST was 67 IU/l, ALT was 64 IU/l, alkaline phosphatase was 430 IU/l and GGT was 429 IU/l. The prothrombin time was 16.7/12.1 s and the partial thromboplastin time was 42.9/26.6 s.

Physical examination demonstrated severe icterus, and a nontender enlarged liver to 14 cm at the mid-clavicular line. There was no splenomegaly, ascites or stigmata of chronic liver disease. There was diffuse lymphadenopathy and multiple subcutaneous nodules were present on the anterior chest wall and left medial thigh. In addition, the patient developed pruritus associated with a macular erythematous rash on the lower extremities.

An abdominal ultrasound scan showed hepatomegaly with several masses in the liver consistent with metastatic disease. The gall bladder was normal in size and free of stones and the intrahepatic ducts were not dilated. Hepatitis B surface antigen, antibody to hepatitis B surface antigen and antibody to core antigen were all negative, as was a slide test for mononucleosis heterophil antibodies (Monospot test). Tests for the IgG antibody to hepatitis A were positive but the IgM result was negative, as were viral serologies for herpes simplex virus I and herpes simplex virus II. A test for the Epstein-Barr virus nuclear antigen was positive 1:8, as was the IgM titer. The IgG titer was 1:10 (weakly positive).

Therapy was complicated by elevated temperatures to 40.5 °C, hypotension which required dopamine, renal insufficiency with a peak serum creatinine of 4.0 mg/dl and anemia that was treated with blood transfusions. *S. viridans* septicemia, which responded to antibiotic therapy, occurred during her first hospital course. The patient received medications that included acetominophen and prochlorperazine for the duration of therapy. Immediately upon discontinuation of rIL-2 and LAK therapy, jaundice began to resolve. Twenty days after completion of therapy, liver functions returned to normal (Fig. 4.)

Discussion

The 2 patients described developed severe intrahepatic cholestasis during therapy with rIL-2 and LAK cells. The diagnosis of rIL-2 hepatotoxicity is strongly suggested by the temporal relationship between the development of cholestasis with the start of rIL-2 therapy and improvement in hepatic function with its discontinuation. This is best demonstrated in case II. The patient sustained hepatic injury with jaundice following the use of rIL-2 and LAK and subsequently, 2 months later, became jaundiced when rechallenged with rIL-2.

In our two patients, it is unlikely that other factors were primarily responsible for the transient liver dysfunction. Neither patient had any evidence of biliary obstruction on ultrasound or CT scan. Acute viral hepatitis with types A and B were excluded. In patient II the tests for the Epstein-Barr virus IgM and IgG were positive in low titers only. Although other factors, including sepsis, hypotension, non-A, non-B hepatitis and hepatotoxic drugs, could have contributed to the heaptic dysfunction; it is unlikely that they played a primary pathogenic role. Both patients had elevated bilirubin prior to any blood transfusion. In case I, cholestasis developed prior to starting any supportive medications. Metastatic melanoma to the liver was present only in case II. The limited extent of tumor involvement and the cyclical nature of the jaundice suggested that rIL-2 was the cause of the hepatic dysfunction observed.

Mild to moderate hepatic toxicity has been reported with rIL-2 therapy (Rosenberg et al. 1985, 1987; West et al. 1987; Lotze et al. 1986b). In a recent study by Rosenberg et al. (1987), 157 patients were treated with 180 courses of an intravenous bolus of rIL-2 plus LAK or rIL-2 alone. A bilirubin level greater than

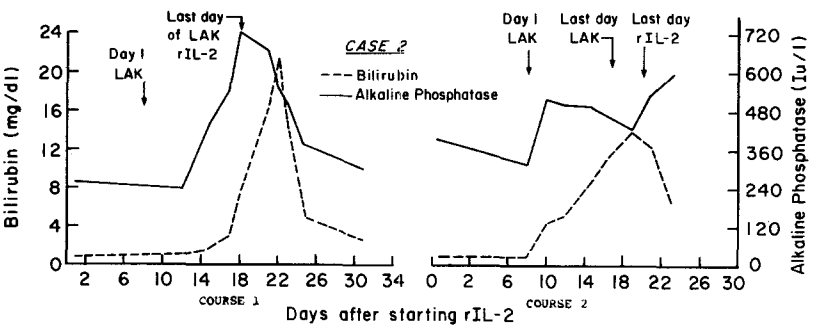

Fig. 4. The development of hyperbilirubinemia and elevated alkaline phosphatase related to rIL-2 and LAK adoptive immunotherapy. After repeat challenge with rIL-2 the patient again had a complete recovery by day 34 (case II)

10 mg/dl developed in 23 of the 180 treatment courses. In that study, 15 patients inadvertently received LAK cells grown in medium containing hepatitis A. This contrasts with an earlier series where only mild transient hyperbilirubinemia was observed (Rosenberg et al. 1985).

West et al. (1987) reported using rIL-2 in a 24-h continuous infusion in 5-day cycles separated by 5-day rest periods (West et al. 1987). The bilirubin level was greater than 3 mg/dl in 5 of the 40 patients (West et al. 1987).

In animal studies, significant elevation of transaminases was found in rats receiving continuous infusion of rIL-2 at doses of $20000\,U\,kg^{-1}\,h^{-1}$ (Matory et al. 1985). Histological examination of the liver revealed periportal lymphocytic infiltrates and hepatocellular necrosis, not unlike those seen in the liver biopsy in case I. The component of rIL-2 administered by both Rosenberg and West included low levels of sodium dodecyl sulfate, which has been shown to cause hepatic toxicity at high doses in mice and rats (Raven et al. 1973; Ikawa et al. 1978; Sohn et al. 1974). However, the rIL-2 administered in this trial did not contain this vehicle. This rIL-2, provided by Hoffman-La Roche, had a molecular mass of 15 kDa and contains 133 amino acids per molecule protein.

Mechanisms of drug-induced hepatic injury include direct hepatotoxicity, host idiosyncrasy or both (Zimmerman 1978). Liver disease may result from hypersensitivity reactions or the production of toxic metabolites, influenced in part by the host's ability to produce or detoxify those metabolites (Zimmerman 1978). In case II, a skin rash was observed, which, in addition to the development of eosinophilia in both cases, suggests a hypersensitivity reaction. The liver biopsy in case I showed cholestasis, periportal inflammation and hepatocellular necrosis, which are non-specific findings, but are consistent with drug-induced hepatotoxicity (Zimmerman 1978) (Figs. 2 and 3).

We should consider interleukin-2 as a drug with the potential to induce severe hepatic injury, characterized primarily by marked cholestasis, which is reversible upon cessation of therapy. Although this toxicity may be related to the schedule used in this trial and the prolonged LAK cell administration. Caution should be exercised when rIL-2 is used in patients with underlying liver disease and when prolonged LAK cell infusion is contemplated. In addition, all patients should be monitored with frequent hepatic function tests during treatment.

References

Atkins MB, Gould JA, Allegretta M et al. (1986) Phase I evaluation of recombinant interleukin-2 in patients with advanced malignant disease. J Clin Oncol 4:1380–1391

Hsieh KH, Shu S, Lee CS, Chu CT, Yang CS, Chang KJ (1987) Lysis of primary hepatic tumors by lymphokine activated killer cells. Gut 28:117–124

Ikawa M, Yoneyama M, Nako T, Hiraya K (1978) Subacute toxicity of detergents: effect of detergents on liver and kidney enzymes in rats. Tokyo-toritsu Kenky, Nempo ISS (Tokyo Prefectural Institute, Annual Report ISS) vol 29 (1), pp 5863–5865

Lotze MT, Robb Rj, Sharrow SO, Frana LW, Rosenberg SA (1984) Systemic administration of interleukin-2 in humans. J Biol Response Mod 3:475–482

Lotze MT, Matory YL, Ettinghausen SE, Rayner AA, Sharrow SO, Seipp CA, Custer M, Rosenberg SA (1985a) In vivo administration of purified human interleukin-2. I. Half life, immunologic effects, and expansion of peripheral lymphoid cells in vivo with recombinant IL-2. J Immunol 135:2865–2875

Lotze MT, Fana LW, Sharrow SO, Robb Rj, Rosenberg SA (1985b) In vivo administration of purified human interleukin-2. I. Half-life and immunologic effects of the jurkat cell line-derived interleukin-2. J Immunol 134:157–166

Lotze MT, Chang AE, Seipp CA, Simson C, Vetto JT, Rosenberg SA (1986a) High-dose recombinant interleukin-2 in the treatment of patients with disseminated cancer: responses, treatment-related morbidity, and histologic findings. JAMA 256:3117–3124

Lotze MT, Matory YL, Rayner AA, Ettinghausen SE, Seipp CA, Rosenberg SA (1986b) Clinical effects and toxicity of interleukin-2 in patients with cancer. Cancer 58:2764–2772

Matory YL, Chang AE, Lipford EH, Braziel R, Hyatt CL, McDonald HD, Rosenberg SA (1985) Toxicity of recombinant human interleukin-2 in rats following intravenous infusion. J Biol Response Mod 4:377–390

Raven H, Schriener H, Kopp O, Peters O (1973) Biochemical environment problems. I. Liver injury by surfactant dodecyl sulphate Arzneimittelforschung 23:134–139

Rosenberg SA, Lotze MT, Muul LM et al. (1985) Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med 313:1485–1492

Rosenberg SA, Lotze MT, Muul LM et al. (1987) A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med 316:889–897

Sohn R, Marinetti G (1974) Effect of detergents on the enzyme activities of the rat liver plasma membrane. Chem Phys Lipids 12:730

West WH, Tauer KW, Yannelli JR, Marshall GD, Orr DW, Thurman GB, Oldham RK (1987) Constant-infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. N Engl J Med 316:898–905

Zimmerman HJ (1978) Hepatotoxicity. Appleton-Century Crofts, New York, pp 91–121

Received October 24, 1988/Accepted December 1, 1988