

# Metabolism and Excretion of Etoposide in Isolated, Perfused Rat Liver Models

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## ABSTRACT

The clearance of etoposide and formation of etoposide glucuronide have been measured in an isolated, perfused rat liver model to evaluate the effect of impaired hepatic function on etoposide kinetics. Hepatocellular injury was produced by pretreatment of rats with allyl alcohol or carbon tetrachloride; ligation of the bile duct simulated obstructive biliary disease. Etoposide clearance ( $3.59 \pm 1.06$  ml/min) was reduced by both carbon tetrachloride ( $2.07 \pm 0.64$  ml/min;  $P = 0.05$ ) and allyl alcohol treatment ( $2.14 \pm 0.62$  ml/min;  $P = 0.05$ ). Biliary obstruction also impaired etoposide clearance but to a lesser extent than hepatocellular injury ( $2.47 \pm 0.69$  ml/min;  $P = 0.20$  versus control). In both hepatocellular and obstructive models, direct biliary etoposide excretion decreased. The metabolic clearance of etoposide to its glucuronide declined by 36% in the hepatotoxin models but was not decreased by biliary obstruction.

Following hepatic injury, there is a reduction in the metabolism and excretion of etoposide by the liver. This effect is most marked on biliary drug excretion. Obstructive biliary disease does not significantly alter etoposide glucuronidation. Since most cancer patients have increased bilirubin on the basis of obstructive disease, little or no etoposide dose alteration will be needed. However, in the patient with significant hepatocellular injury, impaired etoposide clearance will be more pronounced, and etoposide dose alterations may be needed.

## INTRODUCTION

Etoposide, or VP-16-213, is a semisynthetic derivative of podophyllin first synthesized in 1971. It was approved by the Food and Drug Administration for general clinical use in 1984 (1). Etoposide causes tumor cell kill through DNA strand breakage resulting from the interaction of etoposide with the enzyme topoisomerase II and DNA (2). It is widely used in the treatment of testicular cancer, small cell lung cancer, and certain lymphomas (1).

In humans, etoposide has a terminal half-life of 6 to 8 h. Clearance of etoposide occurs via both direct renal excretion and by metabolism. Roughly 35% of an administered dose of drug is excreted into the urine as parent drug (3, 4). Etoposide glucuronide has been identified by mass spectrometry as an etoposide metabolite and accounts for the disposition of 15 to 35% of an administered etoposide dose (5, 6). Impairment of hepatic function could potentially decrease the rate of etoposide elimination. Since parent drug has antineoplastic activity while most metabolites do not (7), impaired elimination would increase the probability of toxicity. At least one current cancer textbook (8) indicates that etoposide dose reductions should be used in patients with severe hepatic dysfunction. This text states, "guidelines for this reduction are not based on solid data."

To determine the role of impaired hepatic function on etoposide clearance, the current study has used the isolated, perfused rat liver model. With this system, pharmacokinetic variables, such as renal clearance and drug distribution, are eliminated. Using this model, etoposide's terminal half-life, its clearance, and the rate of etoposide glucuronide formation have

been measured. Pharmacokinetic parameters found with rat livers pretreated with hepatotoxins and with rat livers in which the biliary tract is obstructed are compared with normal controls.

## MATERIALS AND METHODS

**Materials.** Etoposide was kindly donated by Bristol Laboratories, Wallingford, CT. [<sup>3</sup>H]Etoposide (200 mCi/mmol) was custom synthesized by Moravek Biochemical, Inc., Brea, CA. Purity of radiolabeled drug (>98%) was verified by HPLC<sup>3</sup> analysis in two separate solvent systems. Sprague-Dawley rats (150 to 300 g) were purchased from Harlan-Sprague Dawley, Indianapolis, IN. Solvents for etoposide analysis were HPLC grade and purchased from Fisher Scientific, Memphis, TN.

**Hepatic Perfusions.** Male Sprague-Dawley rats (150 to 300 g) were housed under constant conditions (22°C; 12/12-h light/dark cycle) and maintained on a standard diet (Purina Rat Chow) with water given *ad libitum*. Rats were allocated into one of 3 treatment groups: (a) corn oil given to controls 24 h prior to sacrifice; (b) allyl alcohol (1.8 ml/kg, 1:50 solution made up in normal saline) administered i.v. 24 h before the experiment; and (c) carbon tetrachloride (0.8 mg/kg in corn oil) administered i.p. 24 h prior to the experiment. Livers from rats anesthetized with ether were removed and perfused via the portal vein on an Ambec-Perfusion 1000 apparatus (MX International, Aurora, CO) as described by Evans *et al.* (9). The bile duct was cannulated, and bile was collected in 1-h intervals throughout the experiment. The perfusate was a 20% rat blood and 80% Krebs bicarbonate buffer solution equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> to maintain a pH of 7.4 at 37°C. The volume of the recirculating perfusate was 100 ml, and a constant flow of 20 ml/min was maintained. After 20 min of equilibration, etoposide (2000 µg containing 10.0 µCi of [<sup>3</sup>H]etoposide) was added in a single bolus to the reservoir. Serial perfusate and bile samples were collected over a 180-min period, centrifuged to remove red cells, and frozen at -70°C until assayed. After 180 min, the liver was removed, weighed, homogenized, and stored at -70°C until assayed. There was good viability of all liver preparations over the 3-h perfusion period as assessed by visible inspection and by bile production. Bile volumes ranged from 0.5 to 0.9 ml/h with no significant decrease in production during the 3 h of the experiment.

**HPLC Analysis.** Perfusate and bile samples were analyzed by modification of the HPLC method of Sinkule and Evans (10). To 0.5 ml of perfusate were added 0.5 ml of saturated ammonium sulfate followed by 4 ml of ethyl acetate. Samples were vortexed for 5 min and centrifuged at 3000 rpm × 10 min. The upper organic layer was collected and saved. The aqueous layer was reextracted with 4 ml of ethyl acetate, and the resulting organic layer was combined with the previously collected ethyl acetate layer. The organic extract was evaporated to dryness under a nitrogen stream and reconstituted in 200 µl of methanol. Twenty to 200 µl of this solution were used for HPLC analysis. Bile samples were centrifuged, and 10 to 50 µl were directly injected onto the HPLC column. The HPLC apparatus consisted of a Waters M6000 pump, a Waters 710B injector, and a 4.6 × 250-mm, 10-µm, µBondapak phenyl column (Waters Associates, Milford, MA). Ultraviolet absorbance (254 nm) of the eluate was monitored by a Waters 490 variable wavelength detector coupled to a Waters data module. A mobile phase of water:acetonitrile:acetic acid (74:25:1) separated etoposide from its metabolites. Etoposide elutes from the column with a retention time of 18 min with the glucuronide peaks eluting at 6.3 and 11.2 min (5). Eluate from the column was collected in 0.6 min fractions. Radioactivity in the eluate was determined by adding 5 ml of Aquasol

Received 1/15/88; revised 7/6/88; accepted 7/15/88.

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<sup>2</sup> Supported by NIH Grant CA 39686 and by the Veterans Administration.

<sup>3</sup> The abbreviations used are: HPLC, high-performance liquid chromatography; AUC, area under the concentration-time curve.

(New England Nuclear Corp., Boston, MA) and counting in a liquid scintillation counter using an external standardization procedure to correct for quench.

**Kinetic Calculations.** Half-life ( $t_{1/2}$ ) measurements were estimated using linear regression analysis of the logarithm of the perfusate concentration plotted against time. The volume of distribution ( $V_d$ ) was estimated from the dose ( $D$ ) divided by the perfusate concentration back extrapolated to time zero. Total clearance ( $Cl_t$ ) was calculated from the formula  $Cl_t = V_d \times 0.693/t_{1/2}$ . Partial metabolic clearance was estimated as the total recovered metabolite in perfusate and bile over 180 min divided by the AUC of parent drug (etoposide) from  $t = 0$  to  $t = 180$  min. Biliary clearances of etoposide and etoposide glucuronide were calculated by measuring the amount of compound recovered in bile and dividing by the AUC of that compound in perfusate. Statistical comparisons were made using single-tailed, unpaired Student's  $t$  tests.

## RESULTS

**Elimination of Etoposide by Isolated Perfused Rat Liver Preparations.** To quantitate the rate of hepatic metabolism and etoposide clearance by the normal rat liver,  $^3\text{H}$ -labeled drug (20  $\mu\text{g}/\text{ml}$ ) was perfused into untreated isolated rat liver preparations ( $n = 3$ ). Perfusate samples were collected at timed intervals after the addition of etoposide to the perfusate and injected onto the HPLC column, and the percentage of radioactivity present as glucuronide and parent drug was determined. With time, total radioactivity in the perfusate decreased. By the end of the experiment, nearly all radioactivity represented etoposide glucuronide. Elimination of total radioactivity followed a biexponential curve (Fig. 1). Initial elimination of the tritium label (representing biliary clearance and metabolism of parent drug) was rapid ( $Cl = 2.2 \pm 0.3$  ml/min) with a slower second phase which represents clearance of etoposide glucuronide.

Elimination of parent drug (etoposide) from the perfusate was log linear (Fig. 2). Kinetic parameters for parent drug found following etoposide perfusion of untreated rat liver preparations are shown along the first row of Table 1. The rat liver preparation was efficient in concentrating etoposide with a bile:perfusate concentration ratio of over 100. Nearly all (>98%) of the administered drug was recovered in bile as either parent drug or its conjugate (Fig. 3). The metabolic clearance of etoposide to its glucuronide in control liver preparations was 1.67 ml/min (47% of total clearance), and etoposide biliary clearance was 1.58 ml/min (44% of total clearance) (see Table 2). In this system, 91% of drug clearance could be accounted

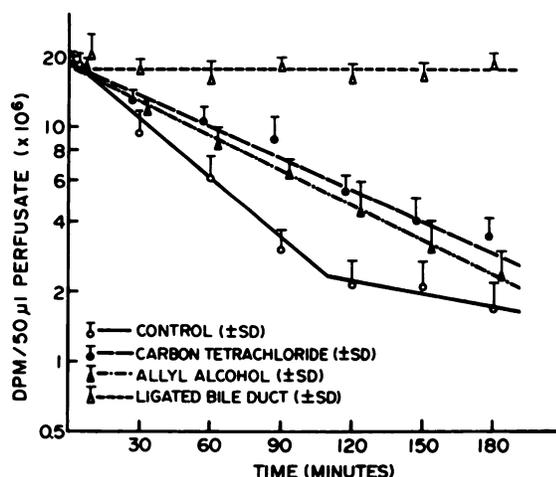


Fig. 1. Clearance of radioactivity from rat liver preparations perfused with [ $^3\text{H}$ ]etoposide. Rats were treated with either corn oil (controls) ( $n = 6$ ), allyl alcohol ( $n = 3$ ), or carbon tetrachloride ( $n = 3$ ) 24 h prior to sacrifice. Three control animals had the bile duct ligated just prior to perfusion to simulate obstruction. dPm/50  $\mu\text{l}$  of perfusate are plotted against time.

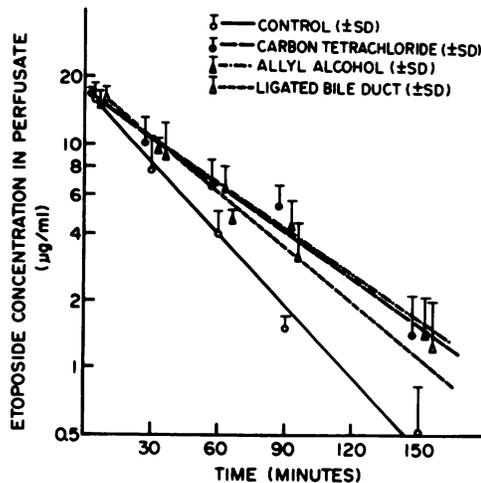


Fig. 2. Clearance of etoposide from rat livers perfused with [ $^3\text{H}$ ]etoposide. Etoposide concentration in the perfusate is plotted against time.

for through biliary excretion of parent drug and metabolism to etoposide glucuronide.

**Kinetics of Etoposide Clearance by Hepatotoxin-treated Isolated Perfused Rat Livers.** Pretreatment with 1.8 ml/kg of a 1:50 solution of allyl alcohol or 0.8 mg/kg of carbon tetrachloride produces histopathological evidence of hepatocellular damage in all rats (11–13). Allyl alcohol produces predominantly periportal necrosis and carbon tetrachloride, predominantly centrilobular, although variations are seen in the extent of the lesion in each individual animal. Treatment with hepatotoxins causes a rise in serum aspartate aminotransferase from  $65 \pm 15$  milliunits/ml to  $1114 \pm 555$  milliunits/ml and decreases the rate of *o*-aminophenol glucuronidation by solubilized hepatic microsomes by 15 to 30% (11–13).

Treatment of rats with either allyl alcohol ( $n = 3$ ) or carbon tetrachloride ( $n = 3$ ) slowed the rate of decrease of tritium from the perfusate (Fig. 1). Clearance of the  $^3\text{H}$ -label was  $2.19 \pm 0.43$  ml/min in control animals versus  $1.28 \pm 0.45$  in carbon tetrachloride-treated animals ( $P = 0.03$  versus control) and  $1.40 \pm 0.36$  in allyl alcohol-treated animals. ( $P = 0.04$  versus control).

Fig. 2 plots the concentration of parent drug (etoposide) in the perfusate as a function of time. Pharmacokinetic analysis of each experiment demonstrated a statistically significant reduced etoposide clearance rate and a prolongation in etoposide half-life with both carbon tetrachloride and allyl alcohol pretreatment (Table 1). The AUC was increased in hepatotoxin-treated animals. Neither hepatotoxin significantly changed the volume of distribution.

Two primary pathways exist in this model for elimination of etoposide: (a) biliary excretion of parent drug and (b) glucuronidation of etoposide. Etoposide glucuronide concentrations in the perfusate peaked 30 to 60 min following initiation of the perfusion and gradually decreased over the next 2 h (Fig. 4). To assess the relative contributions of etoposide excretion into the bile and etoposide glucuronidation to overall etoposide clearance, the concentrations of etoposide and etoposide glucuronide in the bile were measured. Etoposide and etoposide glucuronide present in the liver homogenate at the end of the experiment were also quantitated. Recovery of radioactivity in liver tissue at the end of the experiment was negligible ( $1.8 \pm 0.7\%$  of total administered radioactivity), indicating no sequestration of drug in livers of rats given hepatotoxins. No statistical difference was noted between control and hepatotoxin-treated animals in the percentage of total radioactivity present in the bile at the end of the infusion period, although the percentage of recovery in control bile ( $90.5 \pm 0.8\%$ ) was slightly higher

Table 1 *Etoposide pharmacokinetic measurements in isolated, perfused rat liver preparations*

	AUC ( $\mu\text{g/ml} \times \text{min}$ )	Half-life (min)	Clearance (ml/min)	$V_{\text{dss}}^a$ (ml)
Control	597 $\pm$ 207 <sup>b</sup>	28.9 $\pm$ 2.2	3.59 $\pm$ 1.06	162 $\pm$ 44
Carbon tetrachloride	1030 $\pm$ 321 (0.02) <sup>c</sup>	41.3 $\pm$ 9.1 (0.04)	2.07 $\pm$ 0.64 (0.05)	133 $\pm$ 21 (0.27)
Allyl alcohol	972 $\pm$ 256 (0.06)	43.6 $\pm$ 9.1 (0.01)	2.14 $\pm$ 0.62 (0.05)	143 $\pm$ 21 (0.27)
Ligated bile duct	874 $\pm$ 305 (0.15)	38.3 $\pm$ 5.7 (0.03)	2.47 $\pm$ 0.69 (0.20)	159 $\pm$ 49 (0.47)

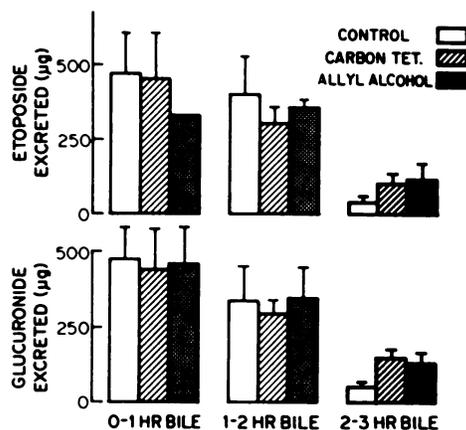
<sup>a</sup>  $V_{\text{dss}}$ , volume of distribution at steady state.<sup>b</sup> Mean  $\pm$  SD.<sup>c</sup> Numbers in parentheses,  $P$  = Student's  $t$  test versus control.

Fig. 3. Biliary excretion of etoposide and etoposide glucuronide into the bile of isolated perfused rat liver preparations. Bile from livers previously treated with corn oil (controls), allyl alcohol, or carbon tetrachloride was collected in 1-h intervals during the perfusion. Concentrations of etoposide and etoposide glucuronide in the bile were measured using HPLC techniques as described in "Materials and Methods."

Table 2 *Mean total, biliary, and metabolic etoposide clearance in ml/min using isolated, perfused rat liver preparations*

	Total clearance	Biliary clearance	Metabolic clearance
Control	3.59	1.58 (44) <sup>a</sup>	1.67 (47)
Carbon tetrachloride	2.07	0.84 (41)	1.04 (50)
Allyl alcohol	2.14	0.84 (39)	1.10 (52)
Ligated bile duct	2.47	0 (0)	2.10 (85)

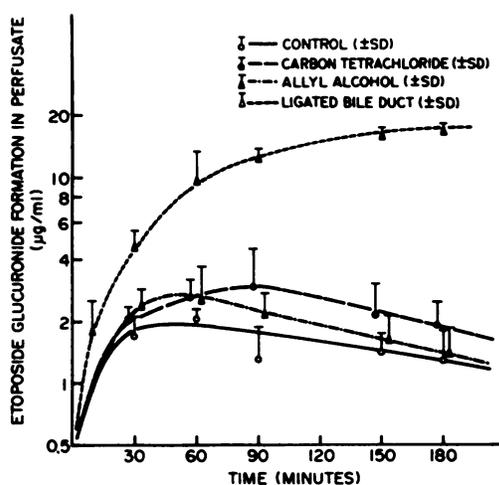
<sup>a</sup> Numbers in parentheses, percentage of mean total clearance.

Fig. 4. Formation of etoposide glucuronide in isolated rat liver preparations perfused with [<sup>3</sup>H]etoposide. Etoposide glucuronide concentration in the perfusate is plotted against time.

than seen in carbon tetrachloride ( $84.3 \pm 2.3\%$ ) or allyl alcohol-treated animals ( $87.4 \pm 3.6\%$ ) ( $P = 0.10$  and  $P = 0.15$ , respectively). The extent of biliary excretion of etoposide and etoposide glucuronide is shown in Fig. 3. Treatment of rats with the hepatotoxins caused a similar percentage of decrease in both

the biliary etoposide clearance and in the partial metabolic clearance of etoposide to its glucuronide metabolite (Table 2). Treatment with hepatotoxins caused a greater reduction in biliary etoposide clearance than in the metabolic clearance.

**Kinetics of Etoposide Clearance in Isolated, Perfused Rat Livers with Ligated Bile Ducts.** The bile ducts of 3 rats were ligated prior to perfusion to approximate acute biliary obstruction. As expected there was no clearance of radioactive tracer from the perfusate using the isolated reperfusion model with the bile duct ligated (Fig. 1). Parent drug was removed at a rate less than that of control animals but greater than that of hepatotoxin-treated rats (Fig. 2; Table 1). Since these animals had their biliary duct ligated, there was no biliary clearance of etoposide. Eighty-five % of etoposide clearance could be accounted for by metabolism to the glucuronide (Table 2). At the end of the 3-h perfusion, etoposide glucuronide comprised over 90% of the radioactivity found in the perfusate (Fig. 4).

## DISCUSSION

Since its introduction for general clinical use, etoposide has become one of the most widely used antineoplastic agents (1). The major toxicity of this drug is myelosuppression which is dose related. At high doses, mucositis is also seen (14). Reducing the rate of drug clearance produces toxicities similar to administering a higher dose of drug. Roughly 30 to 35% of an administered etoposide dose is cleared as parent drug through the kidney, and reduced etoposide doses have been suggested for patients with renal insufficiency (6). Recent studies (5, 15) have demonstrated that hepatic glucuronidation of etoposide is a metabolic elimination pathway. This information has prompted the suggestion for dose reductions in patients with hepatic function impairment (8), although little information has been available which directly addresses this situation.

The isolated, perfused rat liver preparation has been used as a model in this study as controlled hepatobiliary alterations can be produced, and other factors which influence the disposition of a drug in a whole animal, such as renal clearance, drug distribution, and nonhepatic metabolism, can be eliminated or controlled. In our model, hepatocellular toxicity has been simulated through administration of known hepatotoxins. Ligation of the bile duct has been used to approximate obstructive biliary disease. Both simulated disease states, hepatocellular injury and obstructive biliary disease, reduced the clearance and increased the plasma half-life of etoposide. However, the changes were more pronounced in the hepatocellular toxicity model as compared to the model of obstructive biliary disease (42 versus 31% fall in clearance).

Etoposide is cleared from perfused rat livers both by excretion of parent drug into the bile and by glucuronidation. Over 90% of total drug clearance was accounted for by these two processes in our studies. Treatment of animals with hepatotoxins produced less effect on etoposide metabolic clearance than on biliary etoposide excretion. Ligation of the hepatic bile duct leaves hepatocellular function roughly intact. Only a minor

decrease (15%) in the metabolic clearance of etoposide was noted. These results suggest that etoposide glucuronidation is relatively well preserved in acute biliary obstruction and preserved to some extent, even with severe hepatocellular damage. However, both hepatocellular injury and biliary obstruction in the isolated, perfused rat model decrease etoposide biliary excretion.

While these animal studies were being conducted, three groups, including our own, have evaluated the effect of hepatic insufficiency on etoposide clearance in humans (6, 16, 17). Reduced etoposide clearance has, in general, not been found in patients with hepatic insufficiency. These results appear to be at some variance with the perfused liver studies and suggest that, in humans, dose adjustments for hepatic impairment are not needed. However, the strengths and weaknesses of each study must be noted. As noted earlier, many factors may affect the rate of etoposide elimination in patients. Etoposide clearance in humans is likely a result of renal elimination, biliary etoposide clearance, and hepatic metabolism. In both the rat and the human, etoposide glucuronide is a significant metabolite. However, in humans, but not in the perfused liver model, direct renal elimination accounts for 35% of etoposide elimination. Direct biliary excretion of etoposide has accounted for less than 2% of etoposide clearance in the three patients we have studied (18) and in the 3 patients studied by Arbuck (16), whereas it makes a sizable contribution to drug elimination in the isolated perfused rat liver model. Preliminary evidence in our laboratory indicates that the percentage of etoposide excreted by the kidney increases in patients with hyperbilirubinemia (18). In four patients with hepatic obstruction, urinary etoposide excretion accounted for 74% of an administered drug dose, while urinary etoposide excretion accounted for only 30% of an administered dose in 18 patients with normal serum bilirubin. This suggests that the kidney may compensate for impaired hepatic clearance.

Nearly all of the patients with hyperbilirubinemia evaluated in the aforementioned clinical studies have had hepatic function impairment on the basis of obstructive biliary disease. In the perfused rat liver studies, biliary obstruction produced less of an effect on etoposide clearance than did hepatocellular damage. Impairment of etoposide clearance may be more marked in patients with hyperbilirubinemia secondary to hepatitis or other causes of primary hepatocellular toxicity.

In summary, our results indicate that hepatic impairment inhibits both the metabolism and direct excretion of etoposide by the liver. This effect is most marked on direct biliary drug clearance, an elimination pathway used only to a small extent in humans. Impaired etoposide metabolism is more pronounced in livers with hepatocellular damage than in situations of biliary obstruction. The majority of cancer patients with hyperbilirubinemia in whom etoposide will be considered for use will have elevated bilirubin on the basis of obstructive disease secondary to tumor involvement. On the basis of clinical and animal studies, no etoposide reductions need be made for these patients. However, in patients with severe hepatocellular damage or in those patients with concomitant renal function impairment who cannot compensate for impaired biliary clearance, etoposide dose reduction may be needed. Until clinical studies are performed, suggestions for dosing guidelines will be only rough estimates. Based on the studies of D'Incalci (6), Arbuck (16), and Clark (19), we would tentatively suggest a 33% eto-

poside dose reduction for patients with a creatinine clearance of 15 to 25 ml/min/m<sup>2</sup> and a 50% reduction for patients with creatinine clearance less than 15 ml/min/m<sup>2</sup>. In patients who also have obstructive jaundice (bilirubin >2.0 mg/dl), reductions of 50% should be made for patients with a creatinine clearance of 15 to 25 ml/min/m<sup>2</sup>. Etoposide should be avoided in patients with obstructive jaundice and a creatinine clearance less than 15 ml/min/m<sup>2</sup> or in patients with severe hepatitis. These dosage guidelines are only initial suggestions and will need confirmative clinical studies. However, patients with the combination of renal and hepatic failure should be carefully monitored for toxicity if such dose reductions are not made.

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