Cancer Chemother Pharmacol (1986) 16: 43–49

Cancer Chemotherapy and Pharmacology

Effects of advanced leukemia on hepatic drug-metabolizing activity in the mouse

Garth Powis, Richard N. Harris*, Peter J. Basseches, and Kenneth S. Santone**

Division of Developmental Oncology Research, Department of Oncology, Mayo Clinic/Foundation, Rochester, MN 55905, USA

Summary. Mice that had received 10^6 P388 leukemia cells IV 8 days previously exhibited a decrease in the components of the hepatic microsomal mixed function oxidase, with a 58% decrease in cytochrome P-450, and up to a 60% decrease in hepatic microsomal metabolism of biphenyl. Liver weight was increased by 49% due to infiltration of the liver with leukemic cells. Changes in liver drug-metabolizing activity and liver weight were not seen 6 days after administration of P388 leukemia. There was a small increase in serum liver enzyme but no increase in total serum bilirubin in tumor-bearing mice. In vivo total-body plasma clearance of cyclophosphamide, a drug metabolized by hepatic cytochrome P-450, was decreased to 53 ml/min/kg in mice that had received P388 cells 8 days earlier, as against 97.2 ml/min/kg in control mice. Cytochrome P-450-independent metabolism of [^{14}C]5-fluorouracil, measured by means of [^{14}C]CO_2in the breath over 3 h, was decreased to 21% of the dose administered by 8 days after tumor cell administration, compared with 31% of the dose in control mice. P388 leukemia cells growing in the ascitic form in the intraperitoneal cavity of mice did not release an inhibitor of 5-fluorouracil metabolism into the ascitic fluid. Total-body plasma clearance of indocyanine green was decreased to 11 ml/min/kg by 8 days after P388 cell administration, compared with 36 ml/min/kg in control mice. The decrease in indocyanine green clearance might reflect a decrease in hepatic blood flow in the tumor-bearing mice. A possible explanation for the decrease in hepatic drug metabolism caused by P388 leukemia is that the hepatocytes are deprived of oxygen and nutrients by the tumor in the liver, coupled with or caused by a physical obstruction of hepatic blood flow.

Introduction

Studies initiated over 20 years ago by Kato et al. [27] have shown in a variety of animals that primary or transplantable solid tumors growing at a site distant to the liver can produce a decrease in hepatic drug-metabolizing activity [2–9, 12, 15, 20, 25, 28–33, 37, 42, 44–46, 48, 49, 56–58]. The changes include a decrease in the components of the hepatic microsomal mixed function oxidase [7–9, 25, 28, 30–32, 37, 42, 47, 58], a decrease in in vitro hepatic drug-metabolizing activity [2, 3, 6, 7, 9, 12, 20, 27, 28, 30–32, 37, 44, 46, 48, 49, 56–58], and a decrease in in vivo hepatic drug-metabolizing activity [4, 15, 29, 45, 46, 48]. Cyclophosphamide was among the drugs whose metabolism was decreased by extrahepatic tumor [3, 12, 30, 48]. Hepatic drug-metabolizing activity returned to normal when the tumor was surgically removed [5, 20, 46]. Implantation of normal tissue to experimental animals did not decrease hepatic drug-metabolizing activity [45]. These findings were of considerable interest because of the implications of altered drug-metabolizing activity in man. It was shown, however, that significant changes in hepatic drug-metabolizing activity did not occur in animals until the tumor exceeded 2% of the body weight, and large decreases (> 50%) in drug-metabolizing activity were not seen until the tumor equaled or exceeded 10% of the body weight [8, 25, 30, 44–46, 49]. Solid tumors of this size rarely, if ever, occur in man, and it was thus assumed that changes in drug metabolism due to tumor would not be clinically significant in man. This assumption appeared to be confirmed in a study by Tschanz et al. [55], in which no significant differences were found in total body-plasma clearance of antipyrine between lung cancer patients and carefully matched controls.

Recent studies in man have provided circumstantial evidence that solid tumors that metastasize to the liver, although associated with a small tumor burden, can produce a decrease in drug-metabolizing activity [19, 21, 22, 24, 41, 53]. The effect upon hepatic drug-metabolizing activity of tumors that metastasize to or infiltrate the liver has not been studied in animals. A characteristic of many transplantable leukemias is their ability to infiltrate normal tissues, including the liver. The widespread use of murine leukemias, principally P388 and L1210 leukemia, in screening new compounds for antitumor activity and the known activation and inactivation of many drugs by the liver suggest the importance of studying the effects of these tumors on hepatic drug-metabolizing activity.

We report the effects of advanced P388 leukemia in mice on the components of the hepatic microsomal mixed function oxidase and on in vitro drug-metabolizing activity using the test compound biphenyl. The effects of ad-

* *Current address:* Department of Medical Oncology, City of Hope Medical Center, 1500 East Duarte Road, Duarte, CA 91010, USA

** *Current adress:* Ayerst Laboratories Research Inc., Princeton, NJ 08540, USA

Offprint requests to: G. Powis

vanced P388 leukemia on in vivo disposition of cyclophosphamide and 5-fluorouracil and on the plasma clearance of indocyanine green have also been studied.

Methods

Tumor-bearing animals. P388 leukemia cells were obtained from Mason Research Institute, Worcester, Mass, and grown in the ascitic form in male DBA_2 mice. Cells and ascitic fluid were harvested asceptically 7 days after IP injection of 10^6 leukemia cells. For IV injection P388 leukemia cells were washed in 10 mM, pH 7.0, phosphate-buffered 0.9% NaCl, and 10^6 viable cells were injected into the tail vein of male BDF_1 mice weighing 18–23 g (Harlan Sprague-Dawley, Madison, Wis). Mice that had P388 cells injected in this way survived 9–10 days. Some mice received 10^6 P388 leukemia cells IP for in vitro drug metabolism studies. These mice survived 10–12 days and had marked ascites by day 10.

In vitro studies. On days 6 and 8 after the injection of tumor cells groups of six mice were killed by decapitation, and hepatic microsomes were prepared from each animal by differential centrifugation, following homogenization in 0.25 M sucrose according to the method of Ernster et al. [14]. Microsomal cytochrome P-450 levels were determined spectrophotometrically by measuring the difference spectra of the CO complex of dithionite-reduced cytochrome P-450 between 450 and 490 nm [38]. Cytochrome b_5 was determined spectrophotometrically as the difference spectra of dithionite reduced and oxidized cytochrome b_5 between 425 and 410 nm [11]. NADPH-cytochrome P-450 reductase activity was measured by reduction of cytochrome c in the presence of NADPH by the method of Yasukochi and Masters [60].

Biphenyl was used to study the effects of P388 leukemia on hepatic microsomal mixed function oxygenase activity. Biphenyl is a substrate for two species of cytochrome P-450, one inducible by phenobarbital and one by aromatic polycyclic hydrocarbons; the products being 4-hydroxybiphenyl and 2-hydroxybiphenyl, respectively [59]. Biphenyl, 0.1 mM, was added to an incubation mixture containing hepatic microsomes, 1 mg protein/ml, in 1 ml 50 mM potassium phosphate buffer, pH 7.4, 5 mM $MgCl_2$ and an NADPH-generating system consisting of 38 mM glucose-6-phosphate, 0.6 μM $NADP^+$ and 3.5 units/ml glucose-6-phosphate dehydrogenase. The mixture was incubated at 37 °C for 10 min with gentle shaking and the reaction stopped by the addition of 2 ml acetonitrile to precipitate microsomal protein. After standing for 5 min at 4 °C the mixture was centrifuged at 18000 rpm for 2 min in a microcentrifuge (Beckman Instruments, Palo Alto, Calif.). Supernatant, 100 μl, was taken for high-performance liquid chromatography (hplc) on a 25-cm Hibar-II RP-18, 5 μ column (Merck, Darmstadt, West Germany) and eluted isocratically with 56% acetonitrile in 20 mM potassium dihydrogen phosphate at a flow rate of 2 ml/min. The products of biphenyl metabolism were detected with a Schoeffel FS970 fluorometer (Schoeffel Instruments Corp., Westwood, NJ) at an excitation wavelenght of 275 nm and an emission wavelenght of 300 nm.

In vivo drug disposition studies. The effect of advanced P388 leukemia on hepatic microsomal mixed-function oxygenase activity in vivo was measured with the aid of cyclophosphamide, a substrate for cytochrome P-450 [48]. Control mice and mice that had received P388 leukemia 8 days previously received cyclophosphamide, 65 mg/kg IP. Mice were lightly anesthetized with diethyl ether and blood was collected by orbital sinus puncture [39] from groups of six animals at various time points after administration of the cyclophosphamide. Isophosphamide, 2 μg/ml, was added to the plasma as an internal standard. Plasma, 0.5 ml, was mixed with 0.2 ml 1 N sodium hydroxide and extracted with 5 ml ethyl acetate. The ethyl acetate was evaporated under N_2 at 30 °C and the residue derivatized with trifluoroacetic anhydride [26]. Cyclophosphamide was assayed by a gas chromatographic procedure using a 12 M OV-1 capillary column as previously described [18].

The effect of advanced P388 leukemia on noncytochrome P-450 drug metabolism was studied with the aid of 5-fluorouracil [51]. Control mice and mice to which P388 cells had been injected IV 8 days previously received IP injections of [^{14}C-2]5-fluorouracil, 100 mg/kg, 5 μCi. Mice were placed singly in a sealed, all-glass metabolic chambers (Bellacour Co., Laurelton, NJ), and exhaled [^{14}C]CO_2 was collected by bubbling the expired air through 10% KOH. Samples of the KOH, 1 ml each, were taken at 30-min intervals for 3 h and counted by liquid scintillation. Some mice received the hepatotoxin carbon tetrachloride, 0.32 ml/kg, by IP injection with a Hamilton microliter syringe (Hamilton Co., Reno, NV) 24 h before the [^{14}C-2]5-fluorouracil metabolism was investigated. Other mice had daily IP injections for 4 days of cell-free ascites fluid from mice that had received 10^6 P388 leukemia cells IP. Each non-tumor-inoculated mouse received, in succession, the cell-free ascites fluid from four mice that had received P388 leukemia cells IP 6, 7, 8, and 9 days previously. Each recipient mouse was studied 24 h after the last injection of ascites fluid.

Plasma clearance of indocyanine green was used to assess hepatic function and hepatic blood flow. Control and experimental mice received injections into the tail vein of indocyanine green, 10 mg/kg, in 0.1 ml 0.9% NaCl. Groups of four mice were taken at various time points up to 18 min and blood was collected into heparinized tubes by orbital sinus puncture. Recent studies have suggested that the spectrophotometric method commonly used for measuring indocyanine green is nonspecific and may measure a metabolite that is responsible for an apparent second phase of elimination [13]. Preliminary studies have shown that the second phase of elimination was very marked in the mouse. Indocyanine green in plasma was, therefore, measured by a modification of the hplc assay of Rappaport and Thiessen [43]. Briefly, 0.2 ml plasma was mixed with 0.4 ml methanol and allowed to stand at 4 °C for 5 min to precipitate proteins. The mixture was centrifuged at 18000 rpm for 2 min in a microcentrifuge. Supernatant, 100 μl, was injected onto a 25-cm Hibar-II RP-18 5 μ hplc column and eluted with a 10-min gradient of methanol (60%–100%) in 0.3 M sodium acetate, pH 4.0. Eluting compounds were detected at 254 nm. For biliary excretion studies control mice or mice exposed 8 days previously to P388 leukemia were anesthetized with ketamine, 100 mg/kg IP, and the bile duct was cannulated with PE-10 polyethylene tubing (Intramedic, Clay Adams, Parsippany, NJ). Indocyanine green, 5 mg/kg, was injected

through a PE-10 polyethylene cannula in the femoral vein. Bile was collected every 30 min for 3 h into preweighed vials. The bile was diluted 1:100 with water and its optical density measured at 805 nm.

Drugs and chemicals. Biphenyl obtained from Aldrich Chemical Co., Milwaukee, Wis, was purified by thin-layer chromatography on 2-mm silica gel plates developed with toluene. Cyclophosphamide (2H-1, 3, 2-oxaza-phosphorine monohydrate) and 5-fluorouracil were obtained from Sigma Chemical Co., St. Louis, Mo. [^{14}C-2]5-fluorouracil was obtained from ICN Radioisotopes, Irvine, Calif. Indocyanine green was obtained from Hynson, Wescott and Dunning Corp., Baltimore, Md.

Pharmacokinetic and statistical analysis. Plasma drug concentration data was subjected to nonlinear least-squares regression analysis by the NONLIN computer program, and pharmacokinetic parameters calculated [17]. Total-body clearance was calculated as the dose of drug administered divided by the area under the plasma concentration – time curve from time 0 to infinity. Elimination of [^{14}C]CO_2 in the breath from metabolism of [^{14}C]5-fluorouracil was analyzed by the rate-excretion method [17] to obtain first-order rate constants for elimination of parent drug and [^{14}C]CO_2. Groups of data were compared for statistical significance according to Student's t-test [50].

Results

In vitro studies

Mice killed 6 days and 8 days after IV injection of P388 leukemia cells exhibited splenomegaly and mottling of the liver. The mottling was shown histologically to be due to infiltration of the liver with leukemia cells. The degree of mottling was greater in the mice killed on day 8 than in those killed on day 6. Liver weights of mice on day 6 were not significantly different from control values, but on day 8 liver weight had increased by 49% due to tumor (Table 1). Mice killed on day 8 after IV injection of P388 leukemia exhibited decreased levels of hepatic microsomal cytochrome P-450 and b_5 (42% and 79% of control values, respectively), NADPH-cytochrome P-450 reductase activity (65% of control), and microsomal metabolism of biphenyl to 2- and 4-hydroxylated biphenyls (40% and 68% of control, respectively) (Table 1). No change was exhibited in any of these parameters in mice killed on day 6 after IV injection of P388 leukemia (Table 1).

A decrease in hepatic microsomal cytochrome P-450 was also observed in mice that received 10^6 P388 leukemia cells IP. The mean hepatic cytochrome P-450 (nmol/liver: ± SEM) was 22.1 ±0.4 in control mice (n = 5) and 15.8 ±1.1, 8.1 ±0.8 and 8.4 ±0.5 (n = 3, $P < 0.01$ for all 3 values) in mice that had received tumor cells 7, 9, and 10 days previously.

Microsomes prepared from P388 leukemia cells contained no detectable cytochrome P-450, although NADPH-cytochrome P-450 reductase was present at 28.6 ±11.7 nmol/min/mg protein. There was no detectable biphenyl hydroxylase activitiy in microsomes from P388 cells, and biphenyl hydroxylation could not be detected when P388 cells were grown in the presence of 10 μM biphenyl for 24 h (results not shown).

In vivo drug disposition studies

Plasma concentrations of cyclophosphamide in mice in which P388 leukemia had been induced were elevated compared with control mice at all time points up to 75 min after IP administration of the drug (Fig. 1). Plasma elimination of cyclophosphamide was biphasic, with initial and terminal half-lives in control mice of 6.7 min and 34.8 min, and in P388 leukemia-bearing mice of 9.8 and 38.4 min, respectively. Total-body plasma clearance of cyclophosphamide was decreased from 97.2 ml/min/kg in control mice to 53.0 ml/min/kg in mice with induced P388 leukemia.

In vivo metabolism of [^{14}C]5-fluorouracil was measured by elimination of [^{14}C]CO_2 in the breath. Excretion of [^{14}]CO_2 over 3 h was decreased by 33.3% in mice inoculated 8 days previously with P388 leukemia compared with control mice (Fig. 2). A similar decrease was seen in [^{14}C]CO_2 excretion in the breath of mice in which P388 leukemia had been induced by IP injection of cells 8 days

Table 1. Effect of P388 leukemia on hepatic drug metabolizing activity in mice

	Non-tumor-bearing	Tumor-bearing	
		Day 6	Day 8
Liver weight (g)	1.75 ± 0.05	1.66 ± 0.13 (95)	2.61 ± 0.21[a] (149)
Cytochrome P-450 (nmol/liver)	19.2 ± 0.7	17.2 ± 1.8 (90)	8.0 ± 1.2[a] (42)
Cytochrome b_5 (nmol/liver)	4.8 ± 0.2	5.0 ± 0.4 (104)	3.8 ± 0.4[a] (79)
NADPH-Cytocrome P-450 reductase (μmol/min/liver)	2.27 ± 0.04	2.65 ± 0.27 (116)	1.47 ± 0.07[a] (65)
Biphenyl 2-hydroxylase (nmol/min/liver)	8.89 ± 0.68	8.57 ± 0.62 (96)	3.58 ± 0.78[a] (40)
Biphenyl 4-hydroxylase (nmol/min/liver)	26.3 ± 0.6	25.6 ± 1.4 (97)	17.8 ± 2.5[a] (68)

Values are means ± SEM from six animals. Values in parenthesis are percentages of controls.
Values are expressed in terms of total liver activity and are thus independent of changes in liver weight or protein content due to infiltration of the liver by tumor

[a] $P < 0.05$

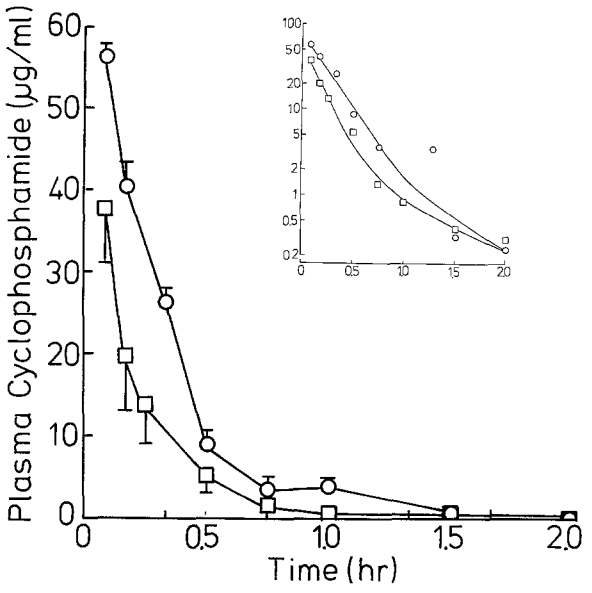

Fig. 1. Effect of P388 leukemia on plasma cyclophosphamide concentrations. Cyclophosphamide was administered IP at a dose of 65 mg/kg to □ non-tumor-bearing mice and ○ mice exposed IV 8 days previously to 10^6 P388 leukemia cells. Each *point* is the mean of 6 mice; *bars*, SEM. *Inset*, semilogarithmic plot of the same data with computer-generated fits to the data shown by the *continuous lines*

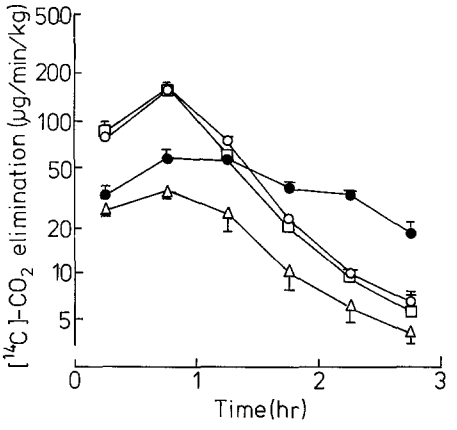

Fig. 2. [^{14}C]CO_2 in breath of mice given [^{14}C-2]5-fluorouracil. [^{14}C-2]5-fluorouracil was administered IP at 100 mg/kg, 5 μCi, to: ○ control mice, n = 5; ● mice exposed IV 8 days previously to 10^6 leukemia P388 cells, n = 4; △ mice pretreated with carbon tetrachloride, 0.32 ml/kg 24 h previously, n = 4; and □ non-tumor-bearing mice that had received IP injections for 4 days of cell-free ascites fluid from P388-bearing mice previously inoculated IP with P388, n = 3. Values are means of n observations and are plotted at the midpoint of the collection period. *Bars*, SEM

previously (results not shown). Carbon tetrachloride, a potent hepatotoxin in mice [18], was used as a positive control to show the effects of liver damage on [^{14}C]5-fluorouracil metabolism. Excretion of [^{14}C]CO_2 in the breath over 3 h was reduced by 69.3% in carbon tetrachloride-pretreated mice compared with control mice. The slopes of the decay phase of the [^{14}C]CO_2 breath elimination curves in Fig. 2 were used to obtain apparent first-order rate constants for elimination of parent [^{14}C]5-fluorouracil (K) and [^{14}C]CO_2 (k_{met}) by the rate-excretion method [17]. The slope of the lines gives either K or k_{met}, whichever is rate-limiting [17]. Direct hplc determination of 5-fluorouracil in plasma of control mice receiving nonlabeled 5-fluorouracil, 100 mg/kg, gave K = 0.099 min^{-1} (these studies are not shown). It appears likely, therefore, that the terminal segment of the excretion time plots for [^{14}C]CO_2 gives k_{met}. This has been assumed in Table 2. P388 leukemia and carbon tetrachloride pretreatment produced significant decreases in K and k_{met}. Ascites fluid taken from mice in which P388 leukemia was induced by IP cell injection, and injected into control mice had no effect on the rate of elimination of [^{14}C]CO_2 (Fig. 2 and Table 2).

Table 2. [^{14}C-2]5-Fluorouracil metabolism to [^{14}C]-CO_2 in the breath of mice

Treatment	*n*	% Excreted as CO_2 in 3 h	k_{met} min^{-1}	K min^{-1}
Control	5	30.9 ± 1.5	0.028 ± 0.001	0.099 ± 0.11
P388 Tumor	4	20.6 ± 1.8[a]	0.011 ± 0.001[b]	0.050 ± 0.009[a]
CCl_4	4	9.5 ± 1.6[b]	0.019 ± 0.002[a]	0.061 ± 0.004[a]
Ascites fluid	3	30.5 ± 0.9	0.031 ± 0.002	

Values are means ± SEM; *n* is the number of animals. In P388-bearing mice and carbon tetrachloride-treated mice k_{met} and K were calculated by the method of residuals [17] from the data in Fig. 2. K could not be calculated directly for control and ascites treated mice because of insufficient data, but for control mice was measured directly from 5-fluorouracil in plasma

[a] $P < 0.05$; [b] $P < 0.01$, compared with control value

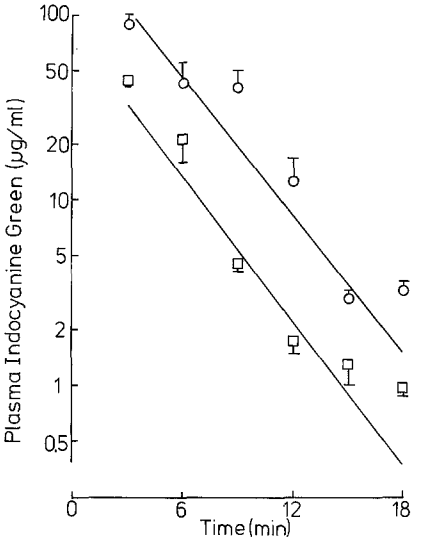

Fig. 3. Effect of P388 leukemia on indocyanine green clearance. Groups of four mice received indocyanine green, 10 mg/kg, into a tail vein. □, non-tumor-bearing mice; ○ mice given IV injections 8 days previously of 10^6 P388 leukemia cells. Values are means ± SEM. *Continous lines* are computer fits to the data

The effect of P388 leukemia injected 8 days previously on plasma elimination of indocyanine green is shown in Fig. 3. Plasma levels of indocyanine green were consistently higher in P388 leukemia-bearing mice than in control mice. Total-body plasma clearance of indocyanine green was 36.0 ml/min/kg in control mice and 11.3 ml/min/kg in P388 leukemia-bearing mice. P388-bearing mice showed a small but nonsignificant ($P > 0.05$) decrease in the excretion of indocyanine green in the bile over 3 h, expressed as a percentage of administered dose with values (± SEM) of 63.4% ±4.3% (n = 7) in tumor-bearing mice,

compared with 74.1 ±3.6 (n = 5) in control mice. The volume of bile secreted over 3 h was 335 ±17 µl in P388 leukemia-bearing mice and 288 ±35 µl in control mice.

Hepatic damage

P388 leukemia produced only moderate signs of liver damage in mice 8 days after IV injection. Serum glutamate pyruvate transaminase activity (± SEM) was increased from 29.1 ± 4.0 IU/l (n = 4) in control mice to 282.7 ± 35.1 (n = 5, $P < 0.01$) in P388-bearing mice. Serum total bilirubin was not significantly elevated, with 0.33 ± 0.07 mg/100 ml in experimental, and 0.24 ± 0.04 mg/100 ml ($P > 0.05$) in control mice.

Discussion

Despite a number of studies on the effects of primary and transplantable solid tumors on hepatic microsomal drug-metabolizing activity [2, 3, 5–9, 12, 20, 25, 27, 28, 30–32, 37, 44, 46, 48, 49, 56–58] and on in vivo drug pharmacokinetics [4, 15, 29, 45, 46, 48] there have been few studies of the effects of transplantable leukemias on drug metabolism and disposition. Lavigne et al. [34] reported an increase in the absorption, and in tissue concentrations of radioactivity from orally administered [^{14}C]cyclophosphamide in mice with advanced intraperitoneal L1210 leukemia. Sonneveld and van Bekkum [52] reported consistently higher serum doxorubicin concentrations in rats with advanced intravenous Brown-Norway myeloid leukemia, but lower doxorubicin concentrations in tissues infiltrated with leukemic cells. Andersson et al. [1] found a marked reduction in the plasma clearance, and a reduced volume of distribution of daunorubicin in rats with advanced intravenous Sutton-August acute non lymphocytic leukemia. It was suggested by Andersson et al. [1] that impaired liver function was only partly responsible for the decreased clearance of daunorubicin in tumor-bearing animals, since serum liver enzymes and bilirubin indicated only moderate liver damage.

In the present study we observed an increase in serum glutamate oxaloacetate transaminase activity but no change in serum total bilirubin concentration in mice that received injections of P388 leukemia IV, despite extensive infiltration of the liver with tumor and a large increase in liver weight. This is similar to the clinical picture of leukemia and lymphoma infiltration of the liver, where in general only small changes in serum liver enzymes and bilirubin are seen [16]. It appears that although P388 leukemia cells infiltrate the liver of mice they do not damage hepatocytes directly.

Despite the absence of signs of marked liver damage mice with advanced leukemia P388, 8 days after IV injection of 10^6 P388 leukemia cells exhibited a decrease in the levels of all the components of the hepatic microsomal mixed-function oxidase. Studies of biphenyl metabolism showed that the activities of at least two forms of cytochrome P-450, phenobarbital-inducible and aromatic hydrocarbon-inducible cytochrome P-450s, were decreased in P388 leukemia-bearing mice. In vivo plasma clearance of cyclophosphamide, a drug that is extensively metabolized by hepatic cytochrome P-450 [48], was decreased 45% in mice with induced P388 leukemia compared with control mice. The liver is an important site of 5-fluorouracil metabolism [10], the rate-limiting step being reduction by cytosolic dihydrouracil dehydrogenase to dihydro-5-fluorouracil [40]. This is followed by conversion to CO_2, urea, ammonia, and a-fluoro-ß-alanine [51]. Metabolism of 5-fluorouracil to CO_2 was inhibited in P388 leukemia-bearing mice with a decrease in the apparent first-order rate constants for elimination of both 5-fluorouracil and CO_2 compared with control mice.

Indocyanine green plasma clearance was decreased 69% in P388 -bearing mice compared with control mice. Indocyanine green clearance has been widely used in man to study hepatic function and to measure liver blood flow [36]. Whether indocyanine green plasma clearance can be taken as a measure of hepatic blood flow in mouse is not known. The value of 36 ml/min/kg obtained in the present study for indocyanine green plasma clearance in non-tumor-bearing mice is close to the reported hepatic plasma flow in rat of 40 ml/min/kg [35]. It is probable that the elimination of indocyanine green in mouse is dependent on hepatic blood flow. If this is the case, an explanation for the decreased indocyanine green clearance in P388-bearing mice could be a decrease in hepatic blood flow. Other mechanisms for the decrease in indocyanine green clearance cannot be ruled out, however. The results of the study, therefore, suggest two mechanisms by which P388 leukemic cell infiltration of the liver might decrease drug elimination. A decrease in the intrinsic drug-metabolizing activity of the liver could lead to a decreased clearance of drugs whose; elimination is capacity-limited, while a decrease in liver blood flow could lead to decreased clearance of a drugs whose elimination is flow-dependent. Only one tumor system has been studied, and whether the results are applicable to other infiltrative tumor systems is not known.

The mechanism for the decrease in hepatic drug metabolism reported with solid tumors has not been elucidated. Blood from solid tumor-bearing rats contains a factor that depresses drug-metabolizing activity of the isolated perfused liver from non-tumor-bearing rats [2, 3, 12] and in non-tumor-bearing rats in vivo [6, 46], but only when the solid tumor grows large enough to become necrotic, suggesting that the release of an inhibitory factor is dependent upon tumor cell death [20, 49]. Solid tumors growing in the ascites form have also been found by some [4, 37, 46], although not all, investigators [49] to depress hepatic drug-metabolizing activity. Toxohormone, a catalase-depressing factor that can be isolated from some solid tumors, has been found in the intraperitoneal fluid associated with ascites tumors [39]. Takahashi and Kato [54] have reported that toxohormone causes a decrease in the activity of the hepatic microsomal mixed-function oxidase similar to that produced by solid tumor. We have found no evidence that ascites fluid from mice with intraperitoneal leukemia P388 contains a factor capable of decreasing the hepatic metabolism of 5-fluorouracil, although we did not study the effects on mixed-function oxidase activity. An alternative explanation for the decrease in hepatic drug-metabolizing activity produced by leukemia P388, and one that finds support from the decreased clearance of indocyanine green, is that liver cells are deprived of oxygen and nutrients by rapidly growing tumor in the liver due to, or coupled with, a physical obstruction of hepatic blood flow.

The implications of the findings of the present study with mice for patients with tumors that infiltrate the liver, e.g., leukemia, neuroblastoma and lymphoma, are not

known. Higuchi et al. [23] reported no difference in the half-life of antipyrine between a small group of patients with acute leukemia prior to treatment and noncancer patients. The patients in this study did not, however, have leukemic cell infiltration of the liver.

In summary, mice in which P388 leukemia had been induced by IV or IP cell injection were found to exhibit a decrease in the components of the hepatic microsomal mixed function oxidase and in the microsomal metabolism of biphenyl at a time when the liver became extensively infiltrated with leukemic cells. There was also a decrease in the in vivo plasma clearance of cyclophosphamide and in the metabolism of 5-fluorouracil to CO_2 at the same time. Serum glutamate oxaloacetate transaminase activity was moderately elevated in tumor-bearing mice, but serum total bilirubin showed no change. Ascites fluid from animals in which tumor had been inoculated IP did not contain a factor that could inhibit 5-fluorouracil metabolism. The plasma clearance of indocyanine green was decreased in P388 leukemia-bearing mice, suggesting that there may be a decrease in the hepatic blood flow in these animals.

Acknowledgements. P.J.B. was supported by an American Leukemia Society Fellowship. The excellent secretarial assistance of Ms Wanda Rhodes in preparing this manuscript is gratefully acknowledged.

References

1. Andersson B, Beran M, Alván G, Peterson C (1981) Differences in the plasma kinetics of daunorubicin in normal and leukemic rats. Acta Radiol Oncol 20: 325–328
2. Bartosek I, Guaitani A, Donelli MG (1972) Prolonged metabolism of pentobarbital in isolated perfused liver of tumor-bearing rats. Biochem Pharmacol 21: 2359–2362
3. Bartosek I, Donelli MG, Guaitani A, Colombo T, Russo R, Garattini S (1975) Differences of cyclophosphamide and 6-mercaptopurine metabolic rates in perfused liver of normal and tumor-bearing animals. Biochem Pharmacol 24: 289–291
4. Beck WT, Mandel HG, Fabro S (1975) Physiological disposition of pentobarbital in tumor-bearing mice. Cancer Res 35: 1333–1340
5. Boulos BM, Sirtori C (1971) Barbiturate metabolism as affected by neoplastic disease. Clin Toxicol 4: 361–368
6. Boulos BM, MacDougall M, Shoeman DW, Azarnoff DL (1972) Evidence that inhibition of hepatic drug oxidation by tumors is mediated by a circulating hormone. Proc Soc Exp Biol Med 139: 1353–1355
7. Brown HD, Chattophadhyay SK, Pennington SN, Spratt JS, Morris HP (1971) Mixed-function oxidation in tumors. Br J Cancer 25: 135–141
8. Chattopadhyay SK, Brown HD, Morris HP (1972) Further studies of electron transport components in a series of Morris hepatoma-bearing rats. Br J Cancer 26: 3–5
9. Collins JM, Dedrick RL, Flessner MF, Guarino AM (1982a) Concentration-dependent disappearance of 5-fluorouracil from pertioneal fluid in the rat: experimental obervations and distribution modeling. J. Pharm Sci 71: 735–738
10. Collins JM, Dedrik RL, Flessner MF, Guarino AM (1982b) Concentration-dependent disappearance of 5-fluorouracil from peritoneal fluid in the rat: experimental observations and distribution modeling. J Pharm Sci 71: 735–738
11. Dallner G (1963) Studies on the structural and enzymic organization of the membranous elements of liver microsomes. Acta Pathol Microbiol Scand [Suppl] 166: 1–80
12. Donelli MG, Bartosek I, Guaitani A, Martini A, Colombo T, Pacciarini MA, Madica R (1976) Importance of pharmacokinetic studies on cyclophosphamide (NSC-26271) in understanding its cytotoxic effect. Cancer Treat Rep 60: 395–401
13. Donn KH, Powell JR, Rogers JF, Plachetka JR (1984) Lack of effect of histamine H^2-receptor antagonists of indocyanine green disposition measured by two methods. J Clin Pharmacol 24: 360–370
14. Ernster L, Siekevitz P, Palade GE (1962) Enzyme-structure relationships in the endoplasmic reticulum of rat liver. J Cell Biol 15: 541–562
15. Franchi G, Rosso R (1969) Metabolic fate of zoxazolamine in tumor-bearing rats. Biochem Pharmacol 18: 236–238
16. Friedman RB, Anderson RE, Entine SM, Hirshberg SB (1980) Effects of disease on clinical laboratory tests. Clin Chem 26 [Suppl]: 1–475
17. Gibaldi M, Perrier D (1982) In: Pharmacokinetics, 2nd edn. Dekker New York
18. Harris RN, Basseches PJ, Appel PL, Durski AM, Powis G (1983) Carbon tetrachloride-induced increase in the antitumor activity of cyclophosphamide in mice: A pharmacokinetic study. Cancer Chemother Pharmacol 12: 167–172
19. Hepner GW, Uhlin SR, Lipton A, Harvey HA, Rohrer GV (1976) Abnormal aminopyrine metabolism in patients with hepatic neoplasm. J Am Med Assoc 236: 1587–1590
20. Hickie RA, Kalant H (1967) Modification of hexobarbital metabolism by Morris hepatoma 5123tc. Can J Physiol Pharmacol 45: 975–983
21. Higuchi T, Nakamura T, Uchino H (1978) Microsomal enzymes in patients with gastric carcinoma as determined by plasma half-life of antipyrine. Gann 69: 753–756
22. Higuchi Z, Nakamura T, Uchino H (1980a) Antipyrine metabolism in cancer patients. Cancer 45: 541–544
23. Higuchi T, Nakamura T, Uchino H (1980b) Microsomal enzymes in patients with acute leukemia as determined by plasma half-life of antipyrine. Cancer Chemother Pharmacol 5: 55–59
24. Higuchi T, Nakamura T, Uchino H (1980c) Antipyrine metabolism in cancer patients. Experientia 36: 236–238
25. Ikeda K, Hozumi M, Sugimura T (1965) Homoproteins in microsomes in liver of tumor-bearing rats. J Biochem (Tokyo) 58: 595–598
26. Juma FP, Rogers HJ, Trounce TR, Bradrook ID (1979) Pharmacokinetics of intravenous cyclophosphamide in man, estimated by gas liquid chromatography. Br J Clin Pharmacol 1: 229–231
27. Kato R (1963) Decreased activities of liver microsomal drug metabolizing enzymes in the rats bearing Walker carcinosarcoma. Experientia 19: 31–32
28. Kato R, Takahasi A (1970) Decreased hydroxylation of seroid hormones by liver microsomes from rats bearing Walker carcinosarcoma 256. Cancer Res 30: 2346–2352
29. Kato R, Takanaka A, Oshima T (1968a) Drug metabolism in tumor-bearung rats: II. In vivo metabolism and effect of drugs in tumor-bearing rats. Jpn J Pharmacol 18: 245–254
30. Kato R, Takanaka A, Takahashi H, Onoda K (1968b) Drug metabolism in tumor-bearing rats: I. Activities of NADPH-linked electron transport and drug-metabolizing enzyme systems in liver micrososmes of tumor-bearing rats. Jpn J Pharmacol 18: 224–244
31. Kato R, Takanaka A, Takahashi A (1970) Decrease in the substrated interaction with cytochrome P-450 in drug hydoxylation by liver microsomes from rats bearing Walker 256 carcinosarcoma. Gann 61: 359–365
32. Kato R, Yamazoe Y, Mita S, Kamataki T, Kubota T, Hanatani Y, Mahejima K (1982) Decrease in the activity of hepatic microsomal drug metabolizing enzymes in tumor-bearing nude mice. Gann 73: 907–911
33. Khandekar JD, Dardachiti D, Garg BD, Tuchweber B, Kovacs K (1972) Hepatic fine structural changes and microsomal hypofunction in Walker tumor-bearing rats. Cancer 29: 738–743
34. Lavigne JG, Barry A, D'Auteuil C, Delage JM (1975) Increase

in the gastrointestinal absorption and in tissue storage of cyclophosphamide in L-1210 leukemic mice at an advanced stage of the disease. Br J Cancer 32: 720–729
35. Lebrec D, Blanchet L, Lacroix S (1981) Measurement of hepatic blood flow in the rat using fractional clearance of indocyanine green and colloidal radiogold. Pflugers Arch 391: 353–354
36. Leevy CM, Mendenhall CL, Lesko W, Howard HM (1962) Estimation of hepatic blood flow with indocyanine green. J Clin Invest 41: 1169–1179
37. Litterst CL, Mimnaugh EG, Gram TE (1977) Effect of strain differences and tumor presence on microsomal drug metabolism in guinea pig. J Natl Cancer Inst 59: 1737–1739
38. Mazel P (1971) Measurement of microsomal drug metabolism In: LaDu BN, Mandel HG, Way EL (eds) Fundamentals of drug metabolism and drug disposition. Williams and Wilkins, Baltimore, pp 546–582
39. Migdalof BH (1976) Methods for obtaining time course data from individual small laboratory animals: Serial microblood sampling and assay, Drug Metab Rev 5: 295–310.
40. Myers CE, Diasio R, Eliot HM, Chabner BA (1976) Pharmacokinetics of the fluoropyrimidines: implications for their clinical use. Cancer Treat Rev 3: 175–183
41. Pelkonen O, Karki NT, Larmi TKI (1973) Liver drug metabolism in tumor-bearing surgical patients. Chir Gastroent 7: 436–443
42. Puccetti P, Giampietri A, Contessa AR (1982) Drug metabolism in experimental cancer immunochemotherapy. Drugs Exp Clin Res 8: 359–366
43. Rappaport PL, Thiessen JJ (1982) High-pressure liquid chromatographic analysis of indocyanine green. J Pharm Sci 71: 157–161
44. Rogers LA, Morris HP, Fouts JR (1967) The effect of phenobarbital on drug metabolic enzyme, activity, ultrastructure and growth of a "minimal deviation" hepatoma (Morris 7800). J Pharmacol Exp Ther 157: 227–244
45. Rosso R, Dolfini E, Donelli MG (1968) Prolonged effect of pentobarbital in tumor-bearing rats. Eur J Cancer 4: 133–135
46. Rosso R, Donelli MG, Franchi G, Garattini S (1971) Impairment of drug metabolism in tumor-bearing animals. Eur J Cancer 7: 565–577
47. Schacter BA, Kurz P (1982) Alterations in hepatic and splenic microsomal electron transport system components, drug metanolism, hemeoxygenase activity, and cytochrome P-450 turnover in Murphy-Sturm lymphosarcoma-bearing rats. Cancer Res 42: 3557–3564
48. Sladek NE (1972) Therapeutic efficacy of cyclophosphamide as a function of its metabolism. Cancer Res 32: 535–542
49. Sladek NE, Domeyer BE, Merriman RL, Brophy GT (1978) Differential effects of Walker 256 carcinosarcoma cells growing subcutaneously, intramuscularly, or intraperitoneally on hepatic microsomal mixed-function oxygenase activity. Drug Metab Dispos 6: 412–417
50. Snedecor GW, Cochran WG (1967) Statistical methods, 6th edn. Iowa State University Press, Ames
51. Sommadossi JP, Gewirtz DA, Diasio RB, Aubert C, Cano JP, Goldman ID (1982) Rapid catabolism of 5-fluorouracil in freshly isolated rat hepatocytes as analyzed by high-performance liquid chromatography. J Biol Chem 257: 8171–8176
52. Sonneveld P, van Bekkum DW (1981) Different distribution of adriamycin in normal and leukemic rats. Br J Cancer 43: 464–470
53. Sotaniemi EO, Pelkonen RO, Mokka RE, Huttunen R, Viljakainen E (1977) Impairment of drug metabolism in patients with liver cancer. Eur J Clin Invest 7: 269–274
54. Takahashi A, Kato R (1969) Effect of toxohormone on microsomal cytochromes and hydroxylating systems of the rat liver. J Biochem 65: 325–327
55. Tschanz C, Hignite CE, Huffman DA, Azarnoff DL (1977) Metabolic disposition of antipyrine in patients with lung cancer. Cancer Res 37: 3881–3886
56. Wilson JT (1968a) An investigation of the decrease in the metabolism of hexobarbital, aminopyrine and p-nitrobenzoic acid by liver from rats bearing a pituitary mammotropic tumor. J Pharmacol Exp Ther 16: 179–188
57. Wilson JT (1968b) Hepatic drug metabolism after phenobarbital or 3-methylcholanthrene pretreatment of rats bearing a pituitary tumor. Proc Soc Exp Biol Med 128: 445–448
58. Wilson JT (1968c) The effect of a pituitary mammotropic tumor on hepatic microsomal drug metabolism in the rat. Biochem Pharmacol 17: 1449–1457
59. Yamazoe Y, Kamatoki T, Kato R (1981) A sensitive method for determination of biphenyl hydoxylase activities using high-performance liquid chromatography. Anal Biochem 111: 126–130
60. Yasukochi Y, Masters BSS (1976) Some properties of a detergent solubilized NADPH-cytochrome c (cytochrome P-450) reductase purified by biospecific affinity chromatography. J Biol Chem 251: 5337–5342

Received February 5, 1985/Accepted May 16, 1985