[CANCER RESEARCH 54, 3723–3725, July 15, 1994]

Advances in Brief

Metabolic Fate of Irinotecan in Humans: Correlation of Glucuronidation with Diarrhea[1]

Elora Gupta, Timothy M. Lestingi, Rosemarie Mick, Jacqueline Ramirez, Everett E. Vokes, and Mark J. Ratain[2]

Section of Hematology/Oncology, Department of Medicine [E. G., T. M. L., R. M., J. R., E. E. V., M. J. R.], Committee on Clinical Pharmacology [R. M., M. J. R.], Cancer Research Center [R. M., E. E. V., M. J. R.]. and Department of Radiation and Cellular Oncology [E. E. V.], University of Chicago Pritzker School of Medicine, Chicago, Illinois 60637

Abstract

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) is hydrolyzed by the enzyme carboxyl esterase to 7-ethyl-10-hydroxycamptothecin (SN-38), which further undergoes glucuronic acid conjugation to form the corresponding SN-38 glucuronide (SN-38G). SN-38 is believed to be the cause of treatment-related diarrhea, a dose-limiting toxicity of CPT-11 observed in phase I clinical trials. This study investigated the effect of glucuronidation on the concentrations of SN-38 following CPT-11 infusion in 21 patients undergoing a phase I trial. To assess the relationship between gastrointestinal toxicity and pharmacokinetics of CPT-11 and its metabolites, we defined a "biliary index" of SN-38 which was the product of the relative area ratio of SN-38 to SN-38G and the total CPT-11 area under the plasma concentration-time curve. Nine patients with grade 3–4 diarrhea had higher biliary indexes than 12 patients with grade 0–2 diarrhea (median 2228 *versus* 5499, $P = 0.0004$). The relatively higher index values, suggestive of higher biliary concentrations of SN-38, were possibly due to low glucuronidation rates. Hence, modulation of glucuronidation may be effective in increasing the therapeutic index of CPT-11.

Introduction

CPT-11[3] is a water-soluble semisynthetic derivative of CPT, a plant alkaloid isolated from *Camptotheca acuminata*. CPT-11 acts as a prodrug *in vivo* and is converted to SN-38 by the enzyme carboxyl esterase (1). SN-38 has been shown to undergo glucuronic acid conjugation to form the corresponding glucuronide which is the major elimination pathway of SN-38 (2). SN-38G is reported to be deconjugated by the intestinal microflora to form SN-38 (3). The topoisomerase I inhibition and single strand breaks after treatment with CPT-11 is determined primarily by SN-38 concentration (4). Accumulation of SN-38 in the intestine was shown to be responsible for the diarrhea attributed to CPT-11 administration in nude mice (5). Thus the *in vivo* activity and toxicity of CPT-11 are dependent on SN-38 concentration, and characterization of the disposition of the metabolite following CPT-11 administration is important for designing optimal dosing schedules. Prior studies have shown inconsistent relationships between the dose or pharmacokinetics of CPT-11 with SN-38 pharmacokinetics and gastrointestinal toxicity (6–9). There have been no reports on the pharmacokinetics of SN-38G in humans. The goals of this study were (*a*) to characterize the plasma profile of SN-38G following CPT-11 administration and (*b*) to estimate the relationship of gastrointestinal toxicity and SN-38 glucuronidation. The complete results of the phase I clinical trial will be reported separately.

Received 5/31/94; accepted 6/15/94.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by Grants N01-CM-07301 and CA-14599.

[2] To whom requests for reprints should be addressed, at University of Chicago, 5841 S. Maryland Ave., MC2115, Chicago, IL 60637.

[3] The abbreviations used are: CPT-11, irinotecan (7-ethyl-10-[4-(1-piperidino)1-piperidino]carbonyloxycamptothecin; CPT, camptothecin; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, SN-38 glucuronide; AUC, area under the plasma concentration-time curve; CL, clearance.

Materials and Methods

Patient Selection, Treatment Plan, and Toxicity Evaluation. Patients with solid tumors or lymphoma were eligible for treatment if they were refractory to standard treatment or if no effective standard treatment existed. All patients had either measurable or evaluable disease, were at least 18 years of age with a Karnofsky's performance status of at least 70%, and had a life expectancy of at least 3 months. All patients met the standard laboratory criteria including criteria for adequate organ function. Informed, written consent was obtained from all patients prior to their first dose of CPT-11. The drug was given in 500 ml normal saline by i.v. infusion over 90 min on a weekly basis for four doses in a 6-week cycle. Weekly dosing was assigned by a standard phase I design using the following dose levels: (*a*) 100 mg/m^2; (*b*) 120 mg/m^2; (*c*) 145 mg/m^2; and (*d*) 175 mg/m^2. Following the first dose, blood and urine sampling was performed for the first 24 h after the infusion for pharmacokinetic evaluations. A second cycle was given with the same dose and schedule used during cycle 1. If dose-limiting toxicity was observed during cycle 1, patients were treated at the previous dose level for all subsequent cycles. Patients who experienced dose-limiting neutropenia were eligible to receive granulocyte colony-stimulating factor at 5 μg/kg/day according to the criteria defined below.

Toxicity assessment was done according to the Cancer and Leukemia Group B expanded toxicity criteria. Patients who experienced ≥grade 2 diarrhea at any time while on study were given loperamide 4 mg p.o. followed by 2 mg p.o. after every stool up to a total dose of 16 mg/day. If loperamide was unsuccessful in controlling diarrhea, treatment was begun with octreotide acetate, 100–600 μg for 2–3 doses/day. Stool collections were also obtained to test for any coexisting infection. CPT-11 doses were withheld until diarrhea resolved to ≤grade 2.

Sample Analysis. To determine drug and metabolite levels, heparinized blood samples obtained on the first cycle of therapy were centrifuged and the plasma was stored at −70°C until analysis. CPT (1 μg/ml, obtained from the National Cancer Institute, Bethesda, MD) was used as an internal standard. One hundred μl of plasma were extracted with 2 ml of methanol and centrifuged at 2500 × *g* for 10 min, and the supernatant was evaporated to dryness. Reconstitution was done with 200 μl of methanol containing 0.1% 10 M HCl (pH ~2.0). For the estimation of SN-38G, plasma samples were extracted as described above. Prior to reconstitution, the samples were incubated with 1000 units of β-glucuronidase (Sigma Chemical Co., St. Louis, MO) for 2 h at 37°C.

The total CPT-11 and SN-38 concentrations in the plasma were estimated by modification of the high performance liquid chromatography method of Barilero *et al.* (10). Analysis was done using a C_{18} column (μBondapak, 10 μm, 3.9 x 300 mm; Waters Associates, Milford, MA) preceded by a C_{18} Novapak guard column. The mobile phase was a mixture of 35% acetonitrile: 65% 0.1 M potassium dihydrogen phosphate containing 3 mM sodium heptane sulfonate (pH 4.0). Detection was monitored by a Hitachi F1050 fluorescence detector (Hitachi Instruments, Inc., Naperville, IL) with a λ_{ex} at 375 nm and λ_{em} at 566 nm. Standard curves of CPT-11 (Yakult Honsha Co., Ltd., Tokyo, Japan) and SN-38 (Yakult Honsha Co., Ltd.) were linear within the range of 5.0–2365.3 ng/ml ($r = 0.99$) and 9.8–116.5 ng/ml ($r = 0.99$), respectively. SN-38G concentrations were determined as the increase in SN-38 concentrations following incubation with β-glucuronidase.

Data Analysis. The plasma concentration-time data of CPT-11, SN-38, and SN-38G were analyzed by noncompartmental analysis using PCNONLIN (SCI, Lexington, KY). The AUC from time zero (predose) to the time of the last

quantifiable concentration (AUC_t) was calculated by the trapezoidal rule. The AUC extrapolated to time infinity ($AUC_{t\to\infty}$) was estimated by dividing the last quantifiable concentration by the terminal rate constant obtained by the log-linear regression of the terminal elimination phase. The AUC was the summation of AUC_t and $AUC_{t\to\infty}$. CL was estimated as the ratio of the dose and AUC.

Since CPT-11-induced diarrhea in nude mice was associated with intestinal accumulation of SN-38 (5), biliary concentrations of the metabolite might be predictive of gastrointestinal toxicity. The principle of area analysis has been used for assessing the disposition of biotransformed drugs (11). The present study used this principle to obtain an estimate of SN-38 excreted in the bile. Since glucuronidation is the major pathway of elimination of SN-38, the fraction of SN-38 not conjugated would be primarily excreted in the bile. The net biliary concentration of SN-38 would then be a resultant of its formation and elimination. This concentration was expressed as the "biliary ratio" which was the ratio of AUC of SN-38 to SN-38G. To control for individual variability in the amount of available drug, the ratio was multiplied by the AUC of CPT-11 to obtain a "biliary index" of SN-38. This was expressed as

$$AUC_{CPT\text{-}11} \times \frac{AUC_{SN\text{-}38}}{AUC_{SN\text{-}38G}}$$

A patient with a low rate of glucuronidation would have relatively higher concentrations of SN-38 in the bile draining into the intestine and would be at a higher risk of gastrointestinal toxicity. Also, patients receiving high doses of CPT-11 may have saturation of the glucuronidation pathway, leading to elevated biliary SN-38 concentrations. Overall, the higher the biliary index of a patient, the greater would be the risk of diarrhea.

The nonparametric Mann-Whitney test was used to test for differences in pharmacokinetic outcomes between two patient groups, defined by the worst severity of diarrhea experienced in the first two cycles of CPT-11 treatment. Statistical tests were performed in the Number Cruncher Statistical System (Dr. Jerry Hintz, Kaysville, UT). A two-sided significance level of ≤ 0.05 was considered statistically significant.

Results and Discussion

Metabolism of CPT-11. Following i.v. infusion of CPT-11, two metabolites could be detected in the plasma, SN-38 and SN-38G. The glucuronide was the major metabolite, with peak plasma concentrations occurring 0.5 to 3 h after the SN-38 peak and plasma levels generally exceeding that of SN-38 (Fig. 1). In addition, a prominent secondary peak was observed in the SN-38 profile (Fig. 1*b*). These observations were in agreement with preclinical studies in rats that reported that 55, 22, and 9% of the biliary radioactivity excreted over 24 h was unchanged CPT-11, SN-38G, and SN-38 and approximately 18% of the biliary radioactivity was reabsorbed from the intestine (2). Pharmacokinetic estimations of the drug and metabolites in the four dose levels are listed in Table 1. There was no effect of pretreatment with G-CSF on the pharmacokinetics of CPT-11 and its metabolites (data not shown). A nonlinear 2.6-fold increase of AUC of CPT-11 from the 100-mg/m^2 to the 175-mg/m^2 dose level correlated to the decrease in CL estimates and was in accordance with previous reports of nonlinear pharmacokinetics of CPT-11 (3, 6, 12). However, there was also a 3.7- and 2.7-fold increase in the AUC estimations of SN-38 and SN-38G, respectively, over the 1.75-fold dose range. Interestingly, there appeared to be no increase in the SN-38G AUC between the 145-mg/m^2 and the 175-mg/m^2 dose levels. The nonlinear increase in CPT-11 AUC seen in the present study could be due to progressive saturation of both the nonmetabolic and metabolic pathways of elimination of CPT-11. The plateau concentrations of SN-38G at the two highest dose levels indicate saturation of glucuronidation of SN-38 to SN-38G. The increase in the SN-38 AUC irrespective of decreasing CL of CPT-11 could be due to the capacity limitation of the glucuronidation pathway of SN-38. The secondary peak in the plasma profile contributing to about a 12% increase in the $AUC_{SN\text{-}38}$ is suggestive of hydrolysis of SN-38G by β-glucuronidase resulting in enterohepatic circulation of SN-38.

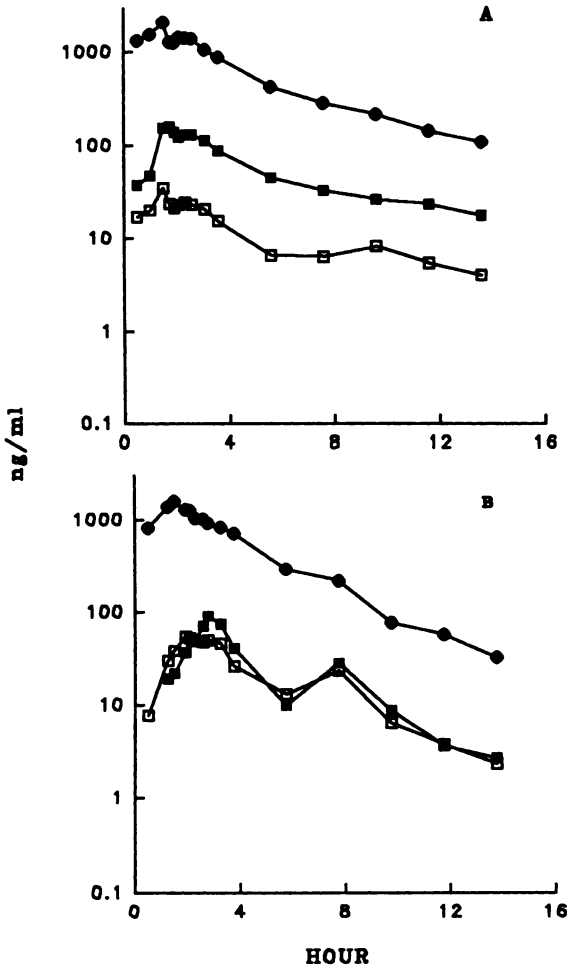

Fig. 1. Plasma disposition curves for CPT-11 (●), SN-38 (□), and SN-38G (■) following i.v. infusion of CPT-11. *A*, representative plasma profile of a patient having grade 0–2 diarrhea; *B*, representative plasma profile of a patient having grade 3–4 diarrhea.

Table 1 *Pharmacokinetic estimates of CPT-11, SN-38, and SN-38G by dose level*

Dose level	$AUC_{CPT\text{-}11}$ ng·h/ml	$AUC_{SN\text{-}38}$ (ng·h/ml)	$AUC_{SN\text{-}38G}$ (ng·h/ml)	CPT-11-CL (liters/h/m^2)
100 mg/m^2 (n= 3)	5,603 ± 967[a]	102.4 ± 28	399.4 ± 344	20.31 ± 4.37
120 mg/m^2 (n=6)	5,031 ± 1,111	127.4 ± 45	268.9 ± 233	24.93 ± 5.98
145 mg/m^2 (n=10)	11,972 ± 6,790	271.2 ± 119	1,152 ± 1,199	13.91 ± 5.98
175 mg/m^2 (n=2)	14,543 ± 5,220	376.1 ± 6.29	1,058 ± 622	12.86 ± 4.62

[a] Mean ± SD.

Interpatient Variability in Disposition. Across dose levels there was a 17–57% variability in the $AUC_{CPT\text{-}11}$ and 2–44% variability in the $AUC_{SN\text{-}38}$ estimates as measured by the percentage coefficient of variation. It has been suggested that variability in CPT-11 disposition was due to interpatient differences in carboxyl esterase levels (6–9). However, estimation of carboxyl esterase activity in predose plasma samples of patients in this study showed poor correlation to dose-normalized AUC of SN-38 or summation of SN-38 and SN-38G (13). This indicated that formation from CPT-11 was not the rate-determining step in the disposition of SN-38. Moreover, on the average 0.25 and 3% of the dose were excreted in the urine as SN-38 and SN-38G, respectively (data not shown). Hence, renal clearance is a minor route of elimination with the major fraction of SN-38 undergoing conjugation and elimination in the bile, an observation consistent with pre-

Table 2 *Correlation of pharmacokinetic estimates to CPT-11 induced diarrhea*

Patients receiving a dose of 145 mg/m^2 were classified according to the worst grade of diarrhea in treatment cycle 1 or 2. Values are represented as median values with the range in parentheses.

Pharmacokinetic estimates	Grade 0–2 (n=5)	Grade 3–4 (n=5)	P
AUC_{CPT-11} (ng·h/ml)	9,160 (8,391–17,918)	14,879 (6,291–23,392)	0.75
AUC_{SN-38} (ng·h/ml)	211.5 (170.0–282.5)	269.1 (161.8–544.5)	0.35
AUC_{SN-38G} (ng·h/ml)	889.9 (413.1–2135)	762.3 (242.4–4206)	0.46
"Biliary ratio"	0.27 (0.12–0.41)	0.53 (0.13–0.87)	0.25
"Biliary index" (ng·h/ml)	2,276 (1,812–3,812)	4,747 (3,028–7,856)	0.03

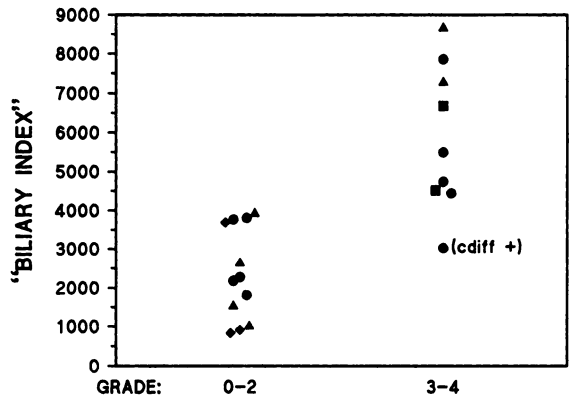

Fig. 2. Scatterplots of biliary index as a function of diarrhea grade at dose levels 100 mg/m^2 (◆), 120 mg/m^2 (▲), 145 mg/m^2 (●), and 175 mg/m^2 (■). Patients were grouped as having either grade 0–2 diarrhea or grade 3–4 diarrhea based on the first two cycles of treatment. The median values for the two groups were 2228 (n = 12) and 5499 (n = 9). One patient with grade 3–4 diarrhea had an index value of 3028, tested positive for *C. difficile* toxin (*cdiff* +). The nonparametric Mann-Whitney test demonstrated a significant correlation of biliary index to severity of diarrhea (P = 0.0004).

vious preclinical reports (2, 3, 12). The interpatient variability (coefficient of variation) in AUC_{SN-38G} across the 4 dose levels ranged from 59 to 104%. Therefore individual differences as well as dose dependency of the SN-38 glucuronidation pathway may be significant and have a major influence on SN-38 disposition. The influence of interpatient variability was reflected in the "biliary ratio" estimates which ranged from 0.15 to 2.53.

Correlation of Diarrhea with Glucuronidation. No diarrhea was observed at the lowest dose level of 100 mg/m^2. There appeared to be a dose-dependent increase in this toxicity with 34, 50, and 100% of patients developing grade 3–4 diarrhea in the 120-mg/m^2, 145-mg/m^2, and 175-mg/m^2 dose levels, respectively. Comparisons of median values of AUC_{CPT-11}, AUC_{SN-38}, and AUC_{SN-38G} and "biliary ratios" between the grade 0–2 and grade 3–4 groups in the 145-mg/m^2 dose level showed no significant differences (Table 2). The only significant variable was the biliary index with median values of 2276 and 4747 for the grade 0–2 and grade 3–4 groups, respectively (P = 0.03). There was also a significant correlation between biliary index and severity of diarrhea based on all dose levels [P = 0.0004 (Fig. 2)]. There was an obvious division of patients based on this index, with about 90% of the patients with grade 3–4 diarrhea having index estimates above 4000. In 4 of 5 patients with grade 3–4 diarrhea in the 145-mg/m^2 dose level, the biliary index was >4000. The fifth patient had an index of 3028 but had a positive stool culture for *Clostridium difficile* toxin (Fig. 2, *cdiff* +) which likely contributed to the severity of diarrhea. Hence, in agreement with our hypothesis, with respect to the total CPT-11 available to the systemic circulation, patients with relatively low glucuronidation rates had progressive accumulation of SN-38 leading to toxicity. The hypothesis was supported by the fact that urinary estimates of the SN-38G were on the average 2.5-fold lower in patients with grade 3–4 diarrhea (data not shown).

Pharmacogenetic variations in drug metabolism have contributed to treatment-related toxicities of several anticancer drugs (14–16). In the case of CPT-11, variability in glucuronidation, which may be genetic, was primarily responsible for differential accumulation of SN-38 in the intestine. Since glucuronidation represents the major detoxification pathway of SN-38, patients deficient in this enzyme activity should have a greater susceptibility to diarrhea. Interindividual differences coupled with interracial differences in glucuronidation have been reported (17). Deficient as well as capacity-limited glucuronosyltransferase activity has been shown to be responsible for the toxicity of drugs such as acetaminophen (18, 19). Therefore, one approach to increasing the therapeutic index of CPT-11 would be to induce glucuronosyltransferase activity.

References

1. Tsuji, T., Kaneda, N., Kado, K., Yokokura, T., Yoshimoto, T., and Tsuru, D. CPT-11 converting enzyme from rat serum: purification and some properties. J. Pharmacobio-Dyn., *14:* 341–349, 1991.
2. Atsumi, R., Suzuki, W., and Hakusui, H. Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion. Xenobiotica, *21:* 1159–1169, 1991.
3. Kaneda, N., Nagata, H., Furuta, T., and Yokokura, T. Metabolism and pharmacokinetics of camptothecin analogue CPT-11 in the mouse. Cancer Res., *50:* 1715–1720, 1990.
4. Kawato, Y., Aonuma, M., Hirota, Y., Kuga, H., and Sato, K. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the anti-tumor effect of CPT-11. Cancer Res., *51:* 4187–4191, 1991.
5. Araki, E., Ishikawa, M., Iigo, M., Koide, T., Itabashi, M., and Hoshi, A. Relationship between development of diarrhea and the concentration of SN-38, an active metabolite of CPT-11, in the intestine and the blood plasma of athymic mice following intraperitoneal administration of CPT-11. Jpn. J. Cancer Res., *84:* 697–702, 1993.
6. Negoro, S., Fukuoka, M., Masuda, N., Takada, M., Kusunoki, Y., Matsui, K., Takifuji, N., Kudoh, S., Niitani, H., and Taguchi, T. Phase I study of weekly intravenous infusion of CPT-11, a new derivative of camptothecin, in the treatment of advanced non-small-cell lung cancer. J. Natl. Cancer Inst., *83:* 1164–1168, 1991.
7. Ohe, Y., Sasaki, Y., Shinkai, T., Eguchi, T., Tamura, T., Kojima, A., Kunikane, H., Okamoto, H., Karato, A., Ohmatsu, H., Kanzawa, F., and Saijo, N. Phase I study and pharmacokinetics of CPT-11 with 5-day continuous infusion. J. Natl. Cancer Inst., *84:* 972–974, 1992.
8. Rothenberg, M. L., Kuhn, J. G., Burris, H. A., III., Nelson, J., Eckardt, J. R., Tristan-Morales, M., Hilsenbeck, S. G., Weiss, G. R., Smith, L. S., Rodriguez, G. I., Rock, M. K., and Van Hoff, D. D. Phase I and pharmacokinetic trial of weekly CPT-11. J. Clin. Oncol., *11:* 2194–2204, 1993.
9. Rowinsky, E. K., Grochow, L. B., Ettinger, D. S., Sartorius, S. E., Lubejko, B. G., Chen, T-L., C., Rock, M. K., and Donehower, R. C. Phase I and pharmacological study of the novel topoisomerase I inhibitor 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) administered as a ninety-minute infusion every 3 weeks. Cancer Res., *54:* 427–436, 1994.
10. Barilero, I., Gandia, D., Armand, J-P., Mathieu-Boue, A., Re, M., Gouyette, A., and Chabot, G. G. Simultaneous determination of the camptothecin analogue CPT-11 and its active metabolite SN-38 by high-performance liquid chromatography: application to plasma pharmacokinetic studies in cancer patients. J. Chromatogr., *575:* 275–280, 1992.
11. Kaplan, S. A., Jack, M. L., Cotler, S., and Alexander, K. Utilization of area under the curve to elucidate the disposition of an extensively biotransformed drug. J. Pharm. Biopharm., *1:* 201–216, 1973.
12. Kaneda, N., and Yokokura, T. Nonlinear pharmacokinetics of CPT-11 in rats. Cancer Res., *50:* 1721–1725, 1990.
13. Gupta, E., Ramirez, J., and Ratain, M. J. Role of carboxyl esterase in the metabolism of CPT-11, a camptothecin analog, in humans. Pharm. Res., In press, 1994.
14. Lennard, L., Van Loon, J. A., and Weinshilboum, R. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. Clin. Pharmacol. Ther., *46:* 149–154, 1989.
15. Harris, B. E., Carpenter, J. T., and Diasio, R. B. Severe 5-fluoro-uracil toxicity secondary to dihydropyrimidine dehydrogenase deficiency: a potentially more common pharmacogenetic syndrome. Cancer (Phila.), *68:* 499–501, 1991.
16. Ratain, M. J., Mick, R., Berezin, F., Janisch, L., Schilsky, R. L., Williams, S. F., and Smiddy, J. Paradoxical relationship between acetylator phenotype and amonafide toxicity. Clin. Pharmacol. Ther., *50:* 573–579, 1991.
17. Patel, M., Tang, B. K., and Kalow, W. Variability of acetaminophen metabolism in Caucasians and Orientals. Pharmacogenetics, *2:* 38–45, 1992.
18. De Morais, S. M. F., and Wells, P. G. Enhanced acetaminophen toxicity in rats with bilirubin glucuronyl transferase deficiency. Hepatology, *10:* 163–167, 1989.
19. Hjelle, J. J. Hepatic UDP-glucuronic acid regulation during acetaminophen biotransformation in rats. J. Pharmacol. Exp. Ther., *237:* 750–756, 1986.