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Report

Absorption of 5'-Deoxy-5-fluorouridine from Colon

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Rectally administered 5'-deoxy-5-fluorouridine (dFUR) is active against transplanted dimethylhydrazine-induced colon tumor in rats. This study investigated the disposition of dFUR in normal non-tumor-bearing rats after rectal administration (350 or 700 mg/kg). An intravenous (iv) bolus injection of [5'-^{3}H]dFUR (28.2 μCi, 0.43 μg) was given 5 min after the rectal dose (700 mg/kg) to determine the dFUR clearance (CL). Blood and fecal samples were analyzed by high-pressure liquid chromatography and liquid scintillation. After the iv tracer dose, the CL was 19 ml/min/kg and the terminal half-life was 50 min. After a 700-mg/kg rectal dose, the terminal half-life was 430 min, the bioavailability was 30%, and the fraction of the dose recovered in 24-hr feces was 34%. After a 350-mg/kg dose, absorption was apparently not completed at 12 hr, as indicated by a lack of decline in blood concentration. The bioavailability of the 350-mg/kg dose exceeded 16%. The absorption of dFUR (700 mg/kg) from the colon was analyzed by the Loo–Riegelman method; the absorption half-life was 550 min. The terminal half-life after the rectal dose was much slower than that after the iv tracer dose but similar to the absorption half-life. These data indicate that dFUR was absorbed from the colon, that the absorption process was the rate-limiting step of its disposition after rectal administration, and that the slow absorption gave a sustained drug concentration in blood.

KEY WORDS: 5'-deoxy-5-fluorouridine; 5-fluorouracil prodrug; rectal absorption.

INTRODUCTION

5'-Deoxy-5-fluorouridine (dFUR)[4] is a metabolic prodrug of 5-fluorouracil (FU) (1,2). Both FU and dFUR are active when given by intravenous (iv) bolus injection or slow infusion. FU is seldom used orally because of its erratic and low bioavailability due to its high hepatic first-pass metabolism (2). In contrast, dFUR is metabolized at a much slower rate by the liver and is absorbed intact from the gastrointestinal tract (3). The oral bioavailability of dFUR is high, ranging from 60% in rats to 80% in man with a relatively small coefficient of variation (<30%) (3,4). Oral dFUR is currently under clinical investigation in Japan. The dose-limiting toxicity of dFUR by this route has been intestinal disturbances (5). Rectal rather than oral administration would circumvent direct exposure of the upper gastrointestinal tract to the drug and may reduce the gastrointestinal toxicity. A study in our laboratory showed that a 7-day rectal treatment with dFUR (700 mg/kg/day) produced 80% cures in rats bearing dimethylhydrazine-induced colon tumors (6). This cure rate is comparable to the results obtained with 7-day regimens of daily iv injection or oral gavage (6,7). The rectal treatment caused less body weight loss than an equally effective oral regimen, suggesting a lower gastrointestinal toxicity. The present study examined the absorption, elimination, and blood concentration–time profile of dFUR and its active metabolite FU after rectal administration to rats.

MATERIALS AND METHODS

Chemicals. dFUR (MW 246.1 g, Lot No. 0305001) and [5'-^{3}H]dFUR (sp act, 66 μCi/μg; Lot No. 133889/54) were provided by Hoffman-La Roche Inc. (Nutley, N.J.). 5-Bromouridine and FU (MW 130.1 g) were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals and solvents were of high-pressure liquid chromatographic (HPLC) or reagent grade and were obtained from Sigma Chemical Co. or Fisher Scientific (Cincinnati, Ohio). HPLC analysis showed that [5'-^{3}H]dFUR was 94% pure and unlabeled dFUR was >99.5% pure.

Animal Protocol. Female Fischer rats (Charles River Breeding Laboratories, Kingston, N.J.) 2–3 months old with a pretreatment weight of 145.7 ± 6.1 g (mean ± SD; $N = 8$) were used. Permanent catheters were implanted in the right jugular veins under ether anesthesia, 1 day before the study. The rats were housed in metabolic cages and had access to water ad lib. Animals were fasted overnight and received two saline enemas at midnight and at 3 hr before treatment. The purpose of administering the enemas was to minimize the feces stored in the colon and therefore reduce the adsorption of dFUR by fecal materials. Animals were allowed access to food 2 hr after treatment ended. A solution of

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[4] Abbreviations used: dFUR, 5'-deoxy-5-fluorouridine; FU, 5-fluorouracil; AUC, area under the concentration–time curve; CL, clearance; HPLC, high-pressure liquid chromatography; iv, intravenous.

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dFUR (140 mg/ml physiologic saline) was infused over 30 min into the colon through a conical plug which delivered the dosing solution at approximately 4 cm from the anus. The plug was constructed using a 200-μl pipette tip (Rainin Instruments, Emeryville, Calif.), a 250-μl Eppendorf centrifugation tube (VWR Scientific, Chicago), and a piece of PE-60 propylethylene tubing (12 cm in length), as described in detail elsewhere (6). Rats received either 350 or 700 mg/kg of dFUR. At 5 min after the 700-mg/kg dose, an iv injection of 30 μCi of [5′-^{3}H]dFUR was administered over 1 min through the venous catheter. The iv tracer dose when corrected for the 94% purity was equivalent to 28.2 μCi. The pH of the drug solution was adjusted to 7 with 1 *N* NaOH. Blood samples were withdrawn through the venous catheter, and serial samples were collected for up to 30 hr. Samples were kept on ice to avoid dFUR degradation by serum phosphorylases.

Sample Analysis. Blood samples were analyzed as described previously (3). In brief, 200 μl blood was mixed with the internal standard 5-bromouridine, extracted with ethyl acetate, and analyzed by HPLC using a reverse phase μBondapak C_{18} column (Waters Associates, Milford, Mass.) and an aqueous mobile phase (pH 5.0) containing 2.5 m*M* ammonium acetate and 1.5% methanol. 5-Bromouridine, dFUR, and FU were detected by their absorbances at 254 and 280 nm. The HPLC fractions containing dFUR were collected and [5′-^{3}H]dFUR was determined by scintillation counting. The maximum [5′-^{3}H]dFUR concentration was <0.2% of the unlabeled dFUR. Hence, the drug concentration quantitated by UV absorbance represented >99% unlabeled dFUR and did not require correction. The standard curves of FU, dFUR, and [5′-^{3}H]dFUR were constructed as described previously (3). Feces samples were soaked overnight with an equal weight of water and homogenized. The aqueous supernatant of the homogenate was extracted with ethyl acetate in the same manner as the blood samples. A separate standard curve of dFUR in feces was constructed.

Data Analysis. The blood concentration–time profile of the iv tracer dose of [5′-^{3}H]dFUR (given concomitantly with the rectal dose) was used to calculate the pharmacokinetics of the drug during the drug absorption from the colon and to establish the pharmacokinetic parameters needed to calculate the absorption rate–time profile. The blood concentration–time data of [5′-^{3}H]dFUR were analyzed using model-independent and -dependent methods. In the model-independent analysis, the area under the drug concentration–time curve (AUC) was calculated by the linear trapezoidal rule, the blood clearance (CL) as dose divided by AUC (8), and the volume of distribution at steady state as the (product of area under the moment curve and dose) divided by (squared AUC) (9). In the model-dependent analysis, the iv [5′-^{3}H]dFUR data were computer-fitted to a two-compartment open model using the NONLIN84 pharmacokinetic data analysis program (10). This program provides the best estimates of AUC, CL, volume of distribution, and rate constants of distribution and elimination. The computer-fitted microconstants of intercompartmental transfer process (k_{12} and k_{21}), the elimination rate constant (k_{el}), and the blood concentration–time data of unlabeled dFUR from the rectal dose were used to calculate the absorption rate–time profile by the Loo–Riegelman analysis (11). The slope of the absorption rate–time profile is the absorption rate constant. We have discussed the application of this method and used it to examine the absorption of dFUR after oral administration in a previous report (3).

The bioavailability of the rectally administered unlabeled dFUR is given by Eq. (1) (8):

$$\text{bioavailability} = \frac{AUC_{rectal} \times CL_{[5'-^3H]dFUR}}{dose_{rectal}} \quad (1)$$

RESULTS

Disposition of iv [5′-^{3}H]dFUR. Figure 1 shows the blood concentration–time curve of [5′-^{3}H]dFUR in five rats given an iv tracer dose of [5′-^{3}H]dFUR at 5 min after receiving a rectal dose of 700 mg/kg. The pharmacokinetic parameters obtained by both model-dependent and -independent analyses are shown in Table I. The good correlation between the observed and the model-predicted values indicates that the disposition of dFUR was well described by a two-compartment open model. The dose and the resulting blood concentrations of [5′-^{3}H]dFUR were <0.2% of those of unlabeled dFUR. Hence, the disposition and metabolism of the rectal dose were not affected by the iv dose, and the CL of [5′-^{3}H]dFUR represented the CL of unlabeled dFUR during rectal absorption. The AUC of [5′-^{3}H]dFUR from 0 to 4 hr was 95.6 ± 2.8% (mean ± SD; range, 92.6 to 99.8%) of the AUC from time 0 to infinity. The dFUR CL was about 19 ml/min/kg, and the elimination half-life was about 50 min.

Disposition of the Rectal dFUR Dose. Figure 2 shows the blood concentration–time profiles of dFUR in rats given 350 mg/kg (*N* = 3) and 700 mg/kg (*N* = 5) of unlabeled dFUR rectally. The maximal dFUR concentrations were 12–19 μg/ml after the 350-mg/kg dose and 22–58 μg/ml after the 700-mg/kg dose. For both doses, the maximal concentrations were reached within 60 min. After the 350-mg dose, the dFUR concentration stayed at a plateau. After the 700-mg dose, the dFUR concentration declined with time; the coefficient of determination (r^2) of the regressed lines of the log-linear terminal phase ranged from 0.831 to 0.986. The termi-

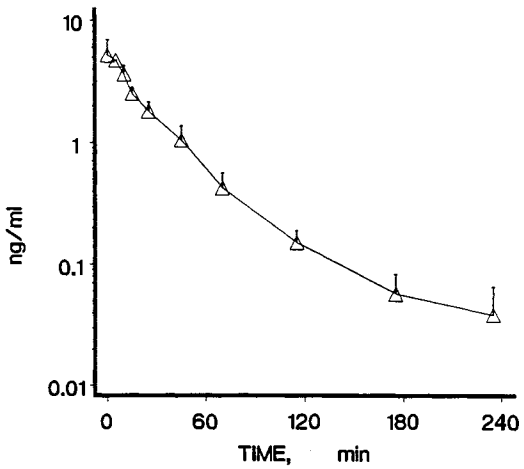

Fig. 1. Blood concentration–time profile of [5′-^{3}H]dFUR in rats given an iv bolus tracer dose of [5′-^{3}H]dFUR (28.2 μCi, equivalent to 0.43 μg). The iv dose was administered 5 min after the rectal infusion of 700 mg/kg of unlabeled dFUR. Mean and one SD (*N* = 5).

Table I. Pharmacokinetic Parameters for [5′-^{3}H]dFUR (28.2 μCi, Equivalent to 0.43 μg) After iv Bolus Injection[a]

Rat No.	AUC (μCi-min/ml)	CL (ml/min/kg)	Volume (ml/kg)	Half-life (min)	k_{12} (min^{-1})	k_{21} (min^{-1})	k_{el} (min^{-1})	r^2
			A. Model-independent analysis					
1	10.082	17.91	900[b]	47	NA	NA	NA	NA
2	8.998	21.25	1713	37	NA	NA	NA	NA
3	8.633	22.43	2586	61	NA	NA	NA	NA
4	12.182	15.50	1247	50	NA	NA	NA	NA
5	11.768	16.46	564	29	NA	NA	NA	NA
Mean	10.333	18.71	1402	45				
SD	1.598	3.01	787	12				
			B. Compartmental analysis					
1	9.715	18.58	601[c]	53	0.00460	0.00934	0.0309	0.998
2	8.328	22.96	708	40	0.00294	0.00203	0.0324	0.962
3	9.319	20.77	621	83	0.01370	0.00442	0.0335	0.992
4	11.331	16.66	658	70	0.00424	0.00434	0.0253	0.982
5	10.960	17.67	429	33	0.02070	0.00525	0.0412	0.998
Mean	9.931	18.93	603	56	0.00920	0.00510	0.0327	0.986
SD	1.226	1.90	106	21	0.00770	0.00270	0.0057	0.015

[a] Data were analyzed by model-independent methods and by compartmental analysis, where data were computer-fitted to a two-compartment open model using the NONLIN84 program. k_{12}, k_{21}, and k_{el} are the microconstants for the intercompartmental transfer and the elimination processes. NA, not applicable. r^2 is the coefficient of determination between the observed and the predicted values.
[b] Volume of distribution at steady state.
[c] Volume of distribution of the central compartment.

nal half-lives ranged from 222 to 690 min, with a mean ± SD of 432 ± 179 min. The corresponding first-order rate constants ranged from 0.003 to 0.001 min^{-1}, with a mean ± SD of 0.00185 ± 0.00008 min^{-1}.

Rectal Bioavailability of dFUR. After the 700-mg/kg dose, the AUC of dFUR from 0 to 24 or 30 hr represented 93.1 ± 6.3% (range, 83.4 to 99.4%) of the calculated AUC from time zero to infinity. The average absolute bioavailability of a 700-mg/kg dose of dFUR, calculated using Eq. (1), was 30% (Table II). The 350-mg/kg dFUR dose gave an AUC of 1.80 ± 0.29 mg-min/ml from 0 to 4 hr and 3.06 ± 1.30 mg-min/ml from 0 to 12 hr. Extrapolation of the AUC to time infinity could not be performed because the dFUR concentrations stayed at a plateau from 4 to 12 hr but declined below the detection limit of 1 μg/ml at the later sampling time

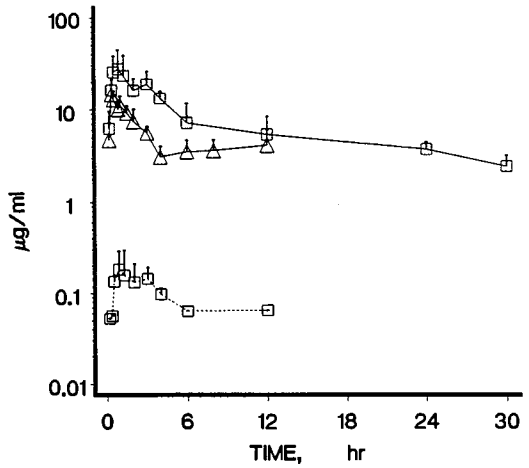

Fig. 2. Blood concentration–time profile of unlabeled dFUR and its active metabolite FU in rats given a 30-min rectal infusion of 350 (△; N = 3) and 700 (□; N = 5) mg/kg of dFUR. dFUR concentrations are solid lines and FU concentrations are dashed lines. The FU concentration after the 350-mg/kg dose were below the detection limit of 50 ng/ml. Mean and one SD.

Table II. Systemic Bioavailability and Fecal Excretion of dFUR After Rectal Administration

Rat No.	AUC (mg-min/ml)	Bioavailability (%)	Fraction of drug excreted in 24-hr feces (%)
	A. 700-mg/kg dose[a]		
1	10.41	26.6	20.5
2	7.04	21.4	22.1
3	8.95	28.7	54.3
4	15.25	33.8	27.1
5	18.01	42.3	51.9
Mean ± SD	11.93 ± 4.56	30.6 ± 7.9	34.0 ± 17.5
	B. 350-mg/kg dose[b]		
1	4.50	23.8	NA[c]
2	1.96	10.4	NA
3	2.73	14.5	NA
Mean ± SD	3.06 ± 1.30	16.2 ± 6.9	NA

[a] AUC was from time 0 to time infinity. Bioavailability was calculated using the drug CL in individual rats.
[b] AUC was from time 0 to 12 hr only. Bioavailability was calculated using the mean CL value of 18.8 ml/min/kg.
[c] Not available.

of 24 hr in most rats. The lower limit of the bioavailability, calculated from the AUC up to 12 hr and the iv clearance of 19 ml/min/kg, was 16%.

Excretion of dFUR in Feces. After the 700-mg/kg rectal dose, the amount of fecal materials excreted in 24 hr was 2.2 ± 2.1 g and the fraction of the dose recovered in the fecal materials was 34% (Table II).

Absorption Rate Profile of dFUR. The absorption rate as a function of time was determined by the Loo–Riegelman method. This method gives the absorption rate–time profile of drugs with two compartment kinetics (11). The results showed that absorption of dFUR was detectable over the entire observation period of 30 hr (Fig. 3). The absorption rate increased during the infusion period and peaked at 30 min. The highest absorption rates occurred at the early time points when the drug concentrations in the colon were highest. The absorption rate–time profile was biexponential, with a rapid absorption phase between 30 min and 2 hr and a slow absorption phase between 2 and 30 hr. The apparent absorption rate constant was 0.0261 ± 0.0181 min^{-1} (corresponding half-life, 41 ± 21 min) during the rapid phase and 0.00146 ± 0.00066 min^{-1} (corresponding half-life, 554 ± 203 min) during the slow phase.

Systemic Availability of FU After dFUR Administration. The FU concentrations were detectable for up to 12 hr after the 700-mg/kg dose but were below the detection limit of 50 ng/ml after the 350-mg/kg dose. After the 700-mg/kg dose, the concentrations of FU rose and declined with the dFUR concentrations (Fig. 2) and were consistently less than 1.5% of the dFUR concentrations. In rats, the half-life of FU is eight times shorter than that of dFUR when administered separately (7). The observed parallelism of FU and dFUR concentrations suggests a formation rate-limited disposition of FU (8), i.e., the formation of FU from dFUR is slower than the elimination of FU. This was shown previously for iv and oral administrations of dFUR (3,7). We used the terminal slope of the concentration–time profile of dFUR to extrapolate the AUC of FU from 4 hr to time infinity. The AUC of FU from time zero to time infinity was 84.4 ± 7.3 μg-min/ml (range, 76.6 to 93.8 μg-min/ml).

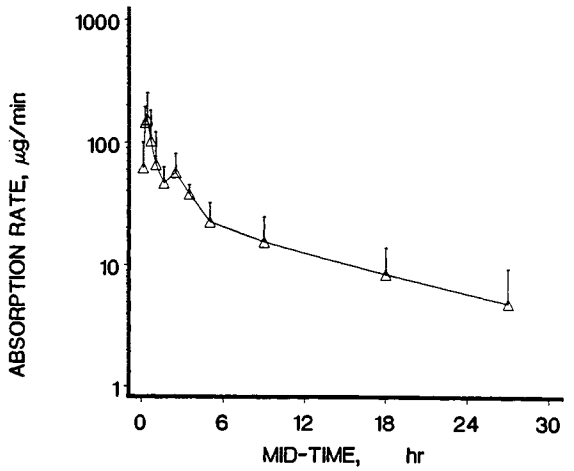

Fig. 3. Absorption rate–time profile of dFUR in rats given a 30-min rectal infusion of 700 mg/kg dFUR. Mean and one SD (N = 5).

DISCUSSION

Data of the present study indicate that dFUR, a fluorinated pyrimidine, was absorbed from the colon. The terminal half-life of the intravenously administered radioactive tracer dose was 50 min, which is shorter than the 2-hr half-life observed after an iv bolus injection of 750 mg/kg (7). In comparison, the half-life of the unlabeled dFUR resulting from rectal administration was much longer at about 7 hr. This indicates a "flip-flop" pharmacokinetic model, where the absorption of dFUR from the colon is the rate-limiting step for its disposition (8). The terminal absorption half-life after the rectal dose was about 9 hr, which is 4.5 times slower than the terminal absorption half-life of 2 hr after an oral dose (3). These data suggest that dFUR is more readily absorbed in the upper gastrointestinal tract than in the colon, consistent with the 10,000-fold greater absorbing surface area in the small intestine compared to the large intestine (12). The bioavailability of dFUR after a 700-mg/kg rectal dose is about one-half of the bioavailability of a 500-mg/kg oral dose (3). The lower bioavailability of the rectal route is most likely due to the slow absorption and loss of drug in feces.

Drugs administered orally and absorbed from the upper gastrointestinal tract are drained into the portal circulation. Drugs administered rectally and absorbed from the lower gastrointestinal tract are drained, in addition to the portal circulation, also into the hemmorhoidal circulation which bypasses the liver and drains directly into the systemic circulation (12). Hence, the first-pass elimination of drugs may be partially avoided by the rectal route (13–15). The ratio of the AUC (molar equivalence) of FU to that of dFUR was 2.1% after the oral dose (3) and 1.3% after the rectal dose. The lower ratio of AUC of FU to AUC of dFUR after the rectal dose suggests a reduced presystemic first-pass metabolism of dFUR to FU.

Seven-day treatments by daily iv bolus injection over 5 min (750 mg/kg), oral gavage (500 mg/kg), and rectal infusion over 30 min (700 mg/kg) produced the same effect against the transplanted dimethylhydrazine-induced colon tumors in rats (3,6,7). But these treatments give different pharmacodynamics, as follows. The AUC of dFUR after the rectal dose (11.93 mg-min/ml) is about 30% of that after the iv injection (43.20 mg-min/ml) (7) or 70% of that after the oral dose (17.34 mg-min/ml) (3). The AUC of the active metabolite FU after the rectal dose (84.4 μg-min/ml) is also only about 40–50% of those after the iv (161 μg-min/ml) and oral doses (192 μg-min/ml) (3,7). These data suggest that the antitumor activity of dFUR is determined by other parameters in addition to the absolute AUC of the drug and its active metabolite. A comparison of the blood concentration–time profiles of dFUR after the three administration routes indicates that a higher dFUR concentration persisted for a longer period of time after the rectal dose. For example, the 24-hr concentration after the rectal dose was 3.65 μg/ml, while those after the oral and iv doses were below the detection limit of 1 μg/ml (3,7). Maximal activity of phase-specific agents such as FU is achieved upon prolonged exposure (16). We speculate that the slow absorption of dFUR from the colon may have provided a prolonged release of the

drug and that the extended drug exposure produced an enhanced effect.

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REFERENCES

1. P. Alberto, M. Rozencweig, M. Clavel, P. Siegenthaler, F. Cavalli, S. Gundersen, U. Bruntsch, J. Renard, and H. Pinedo. *Cancer Chemother. Pharmacol.* **16**:78–79 (1986).
2. B. A. Chabner. In B. A. Chabner (ed.), *Pharmacologic Principles of Cancer Treatment*, W. B. Saunders, Philadelphia, 1982, pp. 183–212.
3. J. L.-S. Au. *J. Pharm. Sci.* **76**:699–702 (1987).
4. R. C. Heinz, T. W. Guentert, C. Sutter, and S. N. Linder-Ciccolunghi. *Proc. Am. Assoc. Cancer Res.* **27**:207 (1986).
5. T. Taguchi, K. Kimura, and T. Saito. *Proc. 3rd Eur. Conf. Clin. Oncol. Cancer Nursing*, 1985, p. 12.
6. S. L. Bramer, L. C. Gunnarsson, and J. L.-S. Au. *Pharm. Res.* **6**:000–000 (1989).
7. J. L.-S. Au, J. S. Walker, and Y. M. Rustum. *J. Pharmacol. Exp. Ther.* **227**:174–180 (1983).
8. M. Rowland and T. N. Tozer. *Clinical Pharmacokinetics: Concepts and Applications*, Lea and Febiger, Philadelphia, 1980.
9. L. Z. Benet and R. L. Galeazzi. *J. Pharm. Sci.* **68**:1071–1074 (1979).
10. Statistical Consultants, Inc. *Am. Stat.* **40**:1 (1986).
11. J. C. K. Loo and S. Riegelman. *J. Pharm. Sci.* **57**:918–928 (1968).
12. A. G. deBoer, F. Molenaar, L. G. J. deLeede, and D. D. Breimer. *Clin. Pharmacokin.* **7**:285–311 (1982).
13. L. G. J. deLeede, A. G. deBoer, G. P. L. M. Rozen, and D. D. Breimer. *J. Pharmacol. Exp. Ther.* **225**:181–185 (1983).
14. L. G. J. deLeede, A. G. deBoer, P. J. M. Havermans, and D. D. Breimer. *Pharm. Res.* **4**:129–141 (1984).
15. A. Kamiya, H. Ogata, and H.-L. Fung. *J. Pharm. Sci.* **71**:621–624 (1982).
16. B. Drewinko and L. Yang. *Cancer Treat. Rep.* **69**:1391–1398 (1985).