[CANCER RESEARCH 44, 4929–4932, November 1984]

Effect of Intravenous Dose and Schedule on Cerebrospinal Fluid Pharmacokinetics of 5-Fluorouracil in the Monkey

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ABSTRACT

There is little information regarding the pharmacology of 5-fluorouracil (5-FUra) in the central nervous system (CNS), despite its role in the treatment of diseases with CNS metastases and recent reports of neurotoxicity. In this study, the plasma and cerebrospinal fluid (CSF) pharmacokinetics of 5-FUra were examined in a primate model. Following a bolus dose, the area under the concentration *versus* time curves for 5-FUra in CSF was 48% of the plasma area under the concentration *versus* time curves. For continuous infusion of 5-FUra, the area under the concentration *versus* time curves ratio for CSF:plasma was 20 or 11%, depending upon the infusion rate. The mechanism for variations in CSF exposure based upon the pattern of plasma delivery is consistent with local metabolism of 5-FUra in the CNS. These findings should be considered in the evaluation of delivery schedules which are intended to maximize drug delivery to the CNS and/or minimize neurotoxicity.

INTRODUCTION

5-FUra[3] is one of the most frequently used agents in oncology. It has demonstrated antineoplastic activity against a wide range of solid tumors, including cancers of the breast, colon, liver, and ovaries (10). Although the plasma pharmacokinetics of 5-FUra has been extensively investigated (5, 6, 9), the pharmacology of this drug in the CNS is largely unknown (2).

There is a substantial rationale for acquiring a greater understanding of the CNS pharmacology of 5-FUra. Recent clinical trials which combine 5-FUra with allopurinol (4) or thymidine (8) report more frequent CNS side effects than with conventional 5-FUra treatment (7, 12). In addition, the more frequent occurrence of CNS metastases in diseases such as breast cancer (11), which are responsive to 5-FUra, suggests that information regarding the disposition of 5-FUra in the CNS would be of value.

In the present study, we examined the CSF and plasma pharmacokinetics of 5-FUra in a primate model. Bolus i.v. and continuous infusion schedules analogous to those used clinically were examined.

MATERIALS AND METHODS

Study Design. Adult male rhesus monkeys (*Macaca mulatta*) were obtained from the NIH Primate Center. All monkeys had s.c. indwelling Ommaya reservoirs, which had been surgically implanted, as described previously (13). This system permits repeated sterile ventricular CSF sampling over extended periods of time in unanesthetized animals.

Three i.v. drug delivery schedules were used. The first simulated the most common delivery of 5-FUra in clinical protocols, 520 mg/sq m given as a bolus. Actual delivery times for a "bolus" vary considerably in clinical settings. In this study, the dose was infused over a 20-min, timed interval. To examine the effect of delivery rate, the same dose (520 mg/sq m) was also given as a 4-hr infusion, *i.e.*, 2.2 mg/sq m/min. Finally, 180 mg/sq m were infused over 4 hr, *i.e.*, 0.75 mg/sq m/min. If continued over a 5-day period, this would correspond to the maximum tolerated continuous infusion rate in humans of 30 mg/kg/day. When feasible, each monkey was used for all experimental schedules, so that interanimal variation would not be a factor.

Blood samples were collected from a separate venous site and centrifuged. Plasma and CSF samples were obtained before the drug was infused, and at hourly intervals during the 4-hr infusions. In addition, plasma samples were taken at 10-min intervals at the end of the infusions and during the 20-min infusions. CSF samples were taken at 15- to 20-min intervals. All samples were frozen at −20° until analyzed.

5-FUra Analysis. Commercially formulated 5-FUra was used for all infusions. 5-FUra and bromouracil for analytical standards were obtained from Sigma Chemical Co. (St. Louis, MO). All solvents were spectral grade.

The 5-FUra assay was a modification of a previously published procedure (3). Bromouracil was added as an internal standard to 0.5-ml samples of plasma and CSF after thawing. Ethyl acetate (4 ml) was added and the sample vortexed. After centrifugation, the ethyl acetate portion was collected and dried under nitrogen at room temperature. The residue was reconstituted with 0.1 ml of buffer (KH_2PO_4, 0.01 M, pH 4.4) and injected onto the column. A Waters Radial Compression Module was used with an 8-mm x 10-cm C_{18} column of 10-μm particle size. The mobile phase consisted of 0.01 M KH_2PO_4 buffer with a methanol gradient of 2 to 50% over 6 min (gradient 9 on Waters Model 660 Solvent Programmer). Flow was 3 ml/min. Absorbance was monitored at both 254 and 280 nm (Waters Model 440). The sensitivity of the assay was 0.1 μM.

Pharmacokinetic Parameters. AUC for 5-FUra in CSF or plasma was calculated by the logarithmic trapezoidal rule from time zero to the last data point. Total AUC was calculated by adding the area calculated from first-order extrapolation from the last data point to infinity. The extrapolated portion was always less than 5% of the total area. Total body clearance was calculated by dividing the total dose administered by the AUC.

RESULTS

Chart 1 displays the 5-FUra concentrations measured in plasma and CSF during and after a 20-min infusion of drug. The plasma concentration peaks at the end of the infusion near 400 μM, then declines with a half-time of about 40 min. There is some indication of nonlinear disappearance, especially for the first hr after the infusion. CSF concentration has peaked by 1 hr at 70 μM, and remains nearly constant for another hr before crossing

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[3] The abbreviations used are: 5-FUra, 5-fluorouracil; CSF, cerebrospinal fluid; CNS, central nervous system; AUC, area under the concentration *versus* time curve.

Received May 21, 1984; accepted August 10, 1984.

the plasma curve at 2 hr and declining at a slower rate than plasma.

Chart 2, *A* and *B*, present the results for 4-hr infusions of 5-FUra. For the infusion rate of 2.2 mg/sq m/min, the plasma concentration reaches its steady-state value of 100 μM by 2 hr. CSF concentration increases throughout the infusion period, and the maximum CSF concentration, 15 μM, occurs at the end of the infusion. At an infusion rate of 0.75 mg/sq m/min, the plasma concentration reaches its steady-state value of 30 μM between 1 and 2 hr. CSF concentration appears to reach a steady-state value, 1.5 μM, between 2 and 3 hr.

Table 1 summarizes the principal pharmacokinetic parameters from the 3 treatment regimens. Since the dose was not the same for all 3 treatment regimens, it is convenient to examine plasma clearance (dose/AUC) to determine the effects of different doses and infusion lengths upon systemic exposure. Plasma clearance varies somewhat, but the differences are less than 2-fold. The AUC for CSF exhibits more pronounced dependence upon the treatment regimen. For a fixed dose of 520 mg/sq m, lengthening the infusion period from 20 to 240 min results in a 4.8-fold decrease in AUC for CSF. When the infusion period is fixed at 4 hr and the dose rate is reduced by a factor of 3, there is an 8.6-fold reduction in AUC for CSF. Although the total dose varies only 3-fold, there is a 42-fold variation in the mean values of absolute CSF exposure for the 3 treatment regimens. The AUC ratio of CSF to plasma can be used to state the relative change in CSF, without focusing on the differences in dose and plasma AUC. This ratio is strongly dependent upon treatment regimen (range, 0.11 to 0.48).

Because of substantial interanimal variation, it is helpful to examine the effects of the treatment regimens in the same

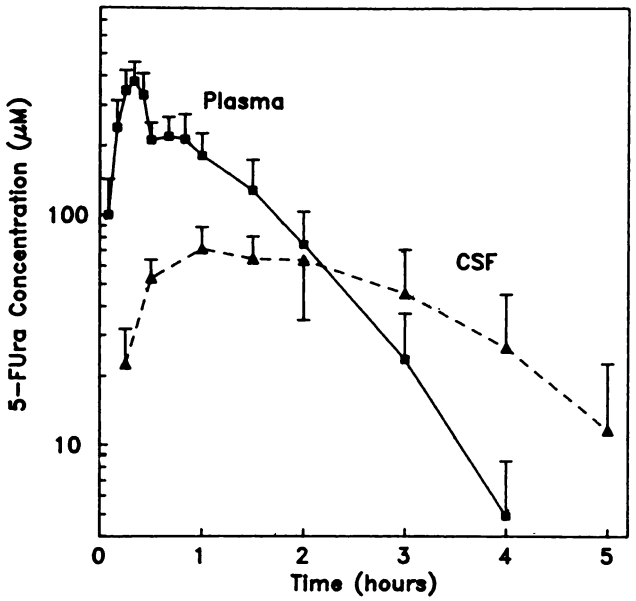

Chart 1. Plasma and CSF concentrations of 5-FUra following a dose of 520 mg/sq m given over 20 min. Data are the arithmetic mean. *Bars*, S.E.

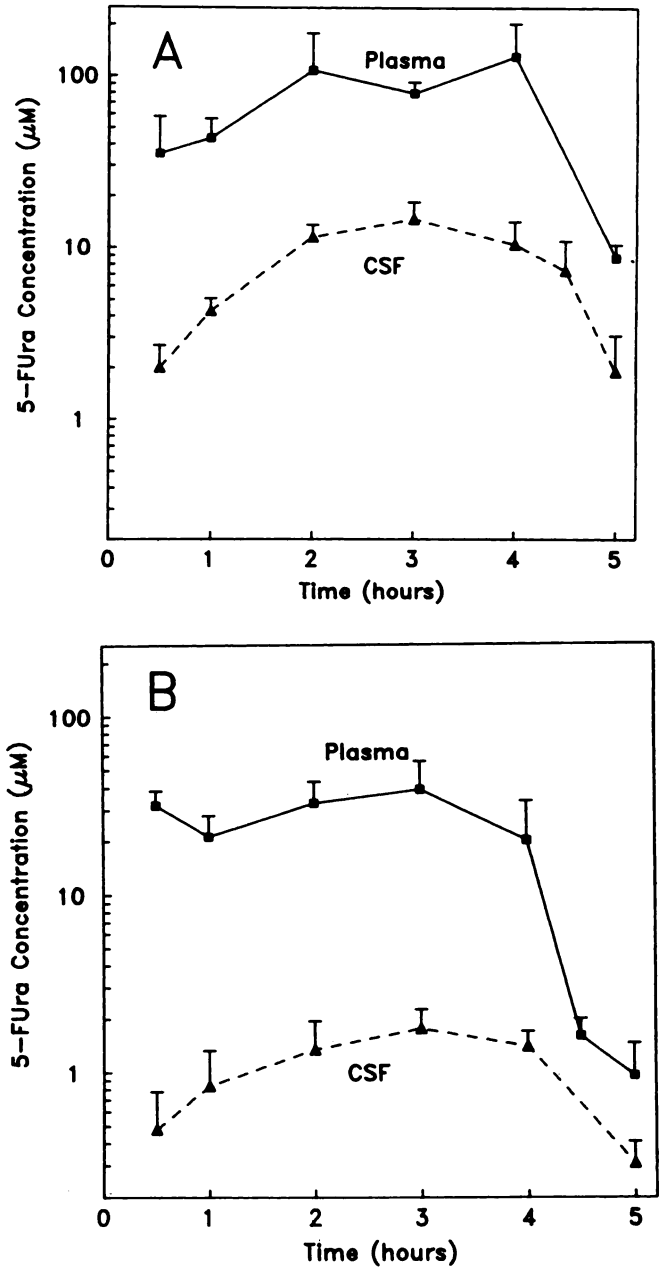

Chart 2. Plasma and CSF concentrations of 5-FUra during and after 4-hr infusions. Data are the arithmetic mean. *Bars*, S.E. *A*, 2.2 mg/sq m/min (total dose, 520 mg/sq m). *B*, 0.75 mg/sq m/min (total dose, 180 mg/sq m).

Table 1

Summary of 5-FUra pharmacology in monkeys

Dose and schedule	Plasma CL (ml/min/sq m)	AUC plasma (μM-hr)	AUC CSF (μM-hr)	AUC ratio (CSF:plasma)
520 mg/sq m bolus (n = 5)	238 ± 67[a]	402 ± 117	221 ± 89	0.48 ± 0.08
520 mg/sq m/4 hr (2.2 mg/sq m/min) (n = 4)	357 ± 150	316 ± 123	48 ± 11	0.20 ± 0.05
180 mg/sq m/4 hr (0.75 mg/sq m/min) (n = 4)	393 ± 206	105 ± 33	5.8 ± 1.8	0.11 ± 0.05

[a] Mean ± S.E.

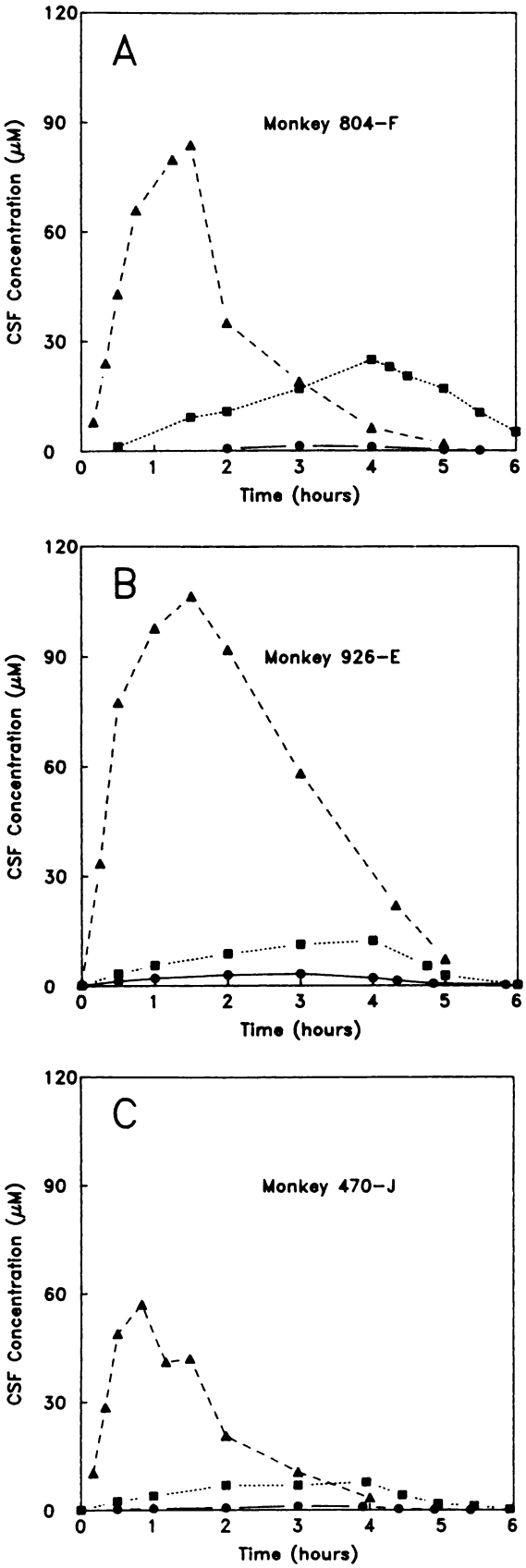

Chart 3. Linear plot of CSF concentrations of 5-FUra for 3 monkeys which were treated with all 3 schedules. ▲, 20-min infusion results; ■, 4-hr infusion of 520 mg/sq m; ●, 4-hr infusion of 180 mg/sq m.

animals. Chart 3, *A* to *C*, display the CSF concentration *versus* time profiles for the 3 individual monkeys who received all 3 treatment regimens. Although semilogarithmic plots are frequently used for pharmacokinetic data, AUC differences are difficult to assess on such plots. To facilitate direct visual comparison of AUC differences, these CSF data are plotted on linear coordinates. For each animal, there is a major difference in total exposure of CSF for the 20-min infusion compared with the 4-hr infusion of the same dose. When the total dose administered over 4 hr is lowered by a factor of 3, the AUC in CSF decreases 5- to 10-fold in these animals.

DISCUSSION

Drug entry into brain and CSF is determined by the permeability of cerebral capillaries as well as plasma drug delivery, *i.e.*, plasma concentration *versus* time (1). Since the pattern of plasma concentration *versus* time is vastly different for various 5-FUra schedules (5, 6, 9), it would be expected that CSF concentration *versus* time would also vary. There are suggestions from the work of Clarkson *et al.* (5) that the CSF variation can't be explained solely on the basis of variations in plasma concentrations. Although not formally calculated by the authors, it appears that the CSF:plasma ratio was 0.25 for a bolus dose in one patient. In another patient, no drug was detectable in the CSF when 5-FUra was given as a continuous infusion.

The qualitative features of 5-FUra pharmacokinetics in the monkey are similar to those observed in humans (6), especially the dependence of plasma AUC upon schedule and the nonlinear increase in plasma AUC with dose. Also, the greater CSF penetration of bolus doses compared with long-term infusions is consistent with isolated observations in humans. There is a quantitative difference in plasma clearance between species. For humans, 5-FUra plasma clearance is about 0.6 liter/sq m/min following a bolus and 3 liters/sq m/min for an infusion (6). In monkeys, the corresponding values are 3- to 8-fold lower, 0.21 liter/sq m/min and 0.35 liter/sq m/min.

The extent of 5-FUra penetration into CSF in the monkey depends upon both schedule and dose. When the infusion time for a fixed dose (520 mg/sq m) is lengthened from 20 min to 4 hr, the AUC in CSF is reduced by 79%. When the 5-FUra dose is reduced by a factor of 3 for a 4-hr infusion, the AUC in CSF is reduced more than 8-fold. When the CSF AUC is corrected for dose reduction, there is a 14-fold range in this exposure parameter. There is a 5-fold range in the relative exposure of CSF to plasma, as expressed by the AUC ratio.

Most of the differences in the CSF AUC are not a result of changes in plasma AUC. There is only a 25% decrease in plasma AUC when the infusion rate is reduced by lengthening the infusion time from 20 min to 4 hr. In addition, there is little difference in plasma AUC when the dose rate is further reduced (corrected for total dose administered). Thus CSF AUC changes are not a passive reflection of total systemic delivery of 5-FUra, but seem to be dependent upon the pattern of 5-FUra delivery.

In addition to differences in mean 5-FUra delivery with dose and schedule, substantial interindividual differences were observed among the monkeys. The plots for individual monkeys (Chart 3) demonstrate this variation, which is also reflected in the large S.E.s seen in Table 1 and Charts 1 and 2.

The CSF AUC differences must relate to nonlinearities in the

mechanisms by which 5-FUra enters and leaves CSF. For example, if carrier systems transported 5-FUra out of CSF, then the higher initial CSF concentrations of 5-FUra would saturate the carriers, prolong the retention of 5-FUra within CSF, and increase the CSF AUC. To our knowledge, there has been no report of carriers for pyrimidines which fits this description. Saturation of influx carriers would have an effect opposite to that observed in this study, namely, the CSF AUC would be greater for the infusions than for the bolus schedule.

Another possibility is concentration-dependent metabolism of 5-FUra within the CNS. This local metabolism could decrease the concentration of 5-FUra as it traverses from capillaries to the CSF. Definitive evidence of CNS metabolism of 5-FUra is not available, but there are indications that 5-FUra is metabolized in many tissues, with a whole-body half-saturating concentration of 15 μM (6). It is interesting to note that the CSF levels of 5-FUra for bolus delivery substantially exceed 15 μM, while the lower continuous infusion rate yields CSF levels an order of magnitude lower. The higher continuous infusion rate yields CSF levels of 5-FUra in the vicinity of 15 μM. Thus, the qualitative features of the CSF concentration *versus* time course are consistent with a mechanism based upon a local metabolism which saturates near 15 μM.

The variation in CSF exposure to 5-FUra should be considered in the selection of treatment regimens. If the treatment goal is to maximize CNS concentrations of 5-FUra, bolus delivery will provide higher CSF AUC than a 24-hr infusion which provides equivalent systemic toxicity. This strategy might be used for a tumor which is metastatic to the CNS (especially meningeal involvement) and is generally sensitive to 5-FUra, such as breast cancer. When there is no tumor suspected in the CNS, a different treatment strategy might be followed. Neurotoxicity is an occasional side-effect of 5-FUra treatment, especially at higher doses (7, 12). The data in this study suggest that it may be desirable to use a prolonged infusion to minimize CNS exposure to 5-FUra.

REFERENCES

1. Blasberg, R. G. Pharmacokinetics and metastatic brain tumor chemotherapy. *In:* L. Weiss, H. A. Gilbert, and J. B. Posner (eds.), Brain Metastases, pp. 146–164. Boston: G. K. Hall and Co., 1980.
2. Bourke, R. S., West, C. R., Chheda, G., and Tower, D. B. Kinetics of entry and distribution of 5-fluorouracil in cerebrospinal fluid and brain following intravenous injection in a primate. Cancer Res., *33:* 1735–1746, 1973.
3. Buckpitt, A. R., and Boyd, M. R. A sensitive method for determination of 5-fluorouracil and 5-fluoro-2′-deoxyuridine in human plasma by high-pressure liquid chromatography. Anal. Biochem., *106:* 432–437, 1980.
4. Campbell, T. N., Howell, S. B., Pfeifle, C., and House, B. A. High-dose allopurinol modulation of 5-FUra toxicity: phase I trial of an outpatient dose schedule. Cancer Treat. Rep., *66:* 1723–1727, 1982.
5. Clarkson, B., O'Connor, A., Winston, L., and Hutchinson, D. The physiologic disposition of 5-fluorouracil and 5-fluoro-2′-deoxyuridine in man. Clin. Pharmacol. Ther., *5:* 581–610, 1964.
6. Collins, J. M., Dedrick, R. L., King, F. G., Speyer, J. L., and Myers, C. E. Nonlinear pharmacokinetic models for 5-fluorouracil in man: intravenous and intraperitoneal routes. Clin. Pharmacol. Ther., *28:* 235–246, 1980.
7. Kaplan, R. S., and Wiernik, P. Neurotoxicity of antineoplastic drugs. Semin. Oncol., *9:* 103–130, 1982.
8. Krook, J., Etzell, P., and Gerstner, J. Potentiation of CNS side effects of 5-fluorouracil (5-FUra) by high dose thymidine. Proc. Am. Assoc. Cancer Res., *24:* 135, 1983.
9. Mukherjee, K. L., Boohar, J., Wentland, D., Ansfield, F. J., and Heidelberger, C. Studies on fluorinated pyrimidines. XVI. Metabolism of 5-fluorouracil-2-C^{14} and 5-fluoro-2′deoxyuridine-2-C^{14} in cancer patients. Cancer Res., *23:* 49–66, 1963.
10. Myers, C. E., Diasio, R., Eliot, H. M., and Chabner, B. A. Pharmacokinetics of the fluoropyrimidines: implications for their clinical use. Cancer Treat. Rev., *3:* 175–183, 1976.
11. Wasserstrom, W. R., Glass, J. P., and Posner, J. B. Diagnosis and treatment of leptomeningeal metastases from solid tumors: experience with 90 patients. Cancer (Phila.), *49:* 759–772, 1982.
12. Weiss, H. D., Walker, M. D., and Weirnik, P. H. Neurotoxicity of commonly used antineoplastic agents. N. Engl. J. Med., *291:* 75–80, 1974.
13. Wood, J. H., Poplack, D. G., Flor, W. G., and Ommaya, A. K. Chronic ventricular cerebrospinal fluid sampling, drug injections and pressure monitoring using subcutaneous reservoirs in monkeys. Neurosurgery *1:* 132–135, 1977.