Europ. J. Cancer Vol. 17, pp. 29–34.
Pergamon Press Ltd. 1981. Printed in Great Britain

0014-2964/81/0101-0029 $02.00/0

Misonidazole Blood and Cerebrospinal Fluid Kinetics in Monkeys Following Intravenous and Intrathecal Administration

DIAMON GANGJI,*‡ DAVID G. POPLACK,§ JAMES SCHWADE,§ JAMES H. WOOD‡ and JOHN M. STRONG||†

‡*Pediatric Oncology Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, U.S.A.*, §*Radiation Oncology Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, U.S.A. and and* ||*Laboratory of Chemical Pharmacology, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, U.S.A.*

Abstract—*Misonidazole (NSC 261037) (RO 070582) blood and cerebrospinal fluid (CSF) levels were studied in the rhesus monkey following* 100 *and* 200 mg/kg *intravenous i.v. and* 0.9 mg/kg *intrathecal i.t. administration.*

After a 200 mg/kg *i.v. dose the plasma half life* (4.8 hr) *was increased in comparison to that following a* 100 mg/kg *dose, suggesting possible saturation of metabolic pathways.*

Levels of desmethylmisonidazole (the major metabolite) were observed in the plasma 5 min *after administration and were approximately* 45% *of those of the parent compound* 4.5 hr *later. Misonidazole and the desmethylated metabolite measured in* 24-hr *urine collections accounted for* 4.8 *and* 19.3% *of the total dose administered, respectively.*

Following i.v. bolus administration misonidazole levels were detected in the CSF at 5 min *and rapidly achieved equilibrium with plasma levels within* 90 min. *Elimination of misonidazole from the CSF occurred at the same rate as that from the plasma. The metabolite was measurably in CSF approximately* 1.5 hr *after administration and ranged from* 33 *to* 82% *of plasma levels.*

Following i.t. injection, misonidazole disappearance occurred in a biexponential manner with mean-disappearance half times of 15 min *and* 38 min *for the fast and slow phases, respectively.*

INTRODUCTION

TUMOR resistance to radiation therapy may, in part, be due to the presence of relatively radioinsensitive hypoxic cells in the tumor. A number of nitroimidazole analogs have been shown to enhance the effect of radiation on hypoxic cells, mimicking the effect of oxygen. Among this class of drugs, the 2-nitroimidazoles have been shown to have a higher radiation enhancement ratio in hypoxic cells than metronidazole, a clinically effective radiation sensitizer [1, 2]. In this group of compounds, misonidazole, 1-(2-nitro-1-imidazolyl)-3-methoxy-2-pronanol (RO 070582), (NSC 261037), has been demonstrated *in vitro* and *in vivo* to be an effective radiosensitizer and is presently undergoing clinical trial [3, 4]. One of the major dose-limiting toxicities encountered in patients has been neurotoxicity (both peripheral and central) which may be related to total dose and time of exposure to the drug [5].

Because of the potential usage of misonidazole in patients with brain tumors and the apparent neurotoxic side effects of this agent, we studied the central nervous system pharmacokinetics of misonidazole in the rhesus monkey.

MATERIALS AND METHODS

Animal model

Recently, an animal model suitable for central nervous system (CNS) pharmacokinetic studies has been described [6]. This

Accepted 3 April 1980.
*Recipient of the Rose et Jean Hoguet Cancer Research Foundation Fellowship, Brussels, Belgium.
†To whom correspondence should be addressed.

model permits sampling of the ventricular fluid under physiologic conditions over extended periods of time without anesthesia. A catheter is inserted into the fourth ventricle and connected to a s.c. Ommaya reservoir. The involved surgical procedure leaves the blood–brain barrier intact. Cerebrospinal fluid (CSF) sampling and i.t. drug administration are performed through the reservoir. Adequate mixing of the ventricular and reservoir CSF fluid was obtained by barbotaging the reservoir [7]. Methotrexate pharmacokinetics after i.t. administration was shown to be similar in this animal system and in humans [8].

Animal studies and sample collection

Three adult male rhesus monkeys (*Macaca mulatta*) weighing 5–6 kg were obtained from the NIH Primate Center. Each animal was fitted with a silicone Pudenz catheter implanted into the fourth ventricle and attached to a s.c. Ommaya reservoir as described [6]. Prior to i.t. injection of misonidazole (0.9 mg/kg) an equivalent amount of CSF was removed in order to maintain a constant ventricular fluid volume. Serial CSF samples were obtained through the Ommaya reservoir after adequate barbotaging. Individual CSF samples never exceeded 0.2 ml and the total CSF removed during individual experiments was never greater than 3 ml. Intravenous administration of misonidazole (100 mg/kg or 200 mg/kg, Table 1) was accomplished using 2-min infusions via a 19 gauge indwelling catheter placed into the femoral vein. CSF samples were immediately frozen and maintained in the dark at −20°C until analyzed. Blood samples were centrifuged, the plasma removed and stored in the dark at −20°C until analyzed. Twenty-four-hour urine collections obtained during three of the experiments were also kept in the dark at −20°C until assayed.

Complete blood counts, liver function tests and blood urea nitrogen (BUN) were obtained prior to each experiment and weekly thereafter for at least three weeks. In one animal (B), a repeat dose was administered at a time when abnormal liver function tests had occurred a few months later (elevated SGOT, SGPT, alkaline phosphatase and LDH). This animal is referred to in Table 1 as animal B' (experiment 3).

Chemical reagents

Reference misonidazole, 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (RO 070582) (NSC 261037), the major known metabolite of misonidazole, desmethylmisonidazole, 1-(2-nitro-1-imidazolyl)-3-hydroxy-2-propanol (RO 059963) (NSC 261036) and a fluorinated analog 1-(2-nitro-1-imidazolyl)-3-fluoro-2-propanol (RO 070791) (NSC 292930) used as the internal standard for quantitation were obtained from the Developmental Therapeutics Program of the Division of Cancer Treatment, National Cancer Institute. Misonidazole (25 mg/ml in 0.5 N saline solution) used for i.v. administration was generously supplied by W. E. Scott, Hoffman La Roche; Nutley, N.J. *N,O-bis*-trimethylsilyltrifluoroacetamide (BSTFA) used for derivatizing the compounds was purchased from the Pierce Chemical Co., Rockford, Ill. 1-(2-nitro-2-^{14}C-1-imidazolyl)-3-methoxy-2-propanol used for protein binding studies was supplied by SRI International, Contract N01-CM-63776, Menlo Park, Calif.

Protein binding studies

The percentage protein binding of misonidazole to bovine serum albumin was evaluated using standard dialysis techniques. A 1-ml aliquot of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M solution of ^{14}C-misonidazole and 4.6×10^{-7} M bovine serum albumin was dialyzed against 20 ml of a phosphate buffered saline solution pH 7.5. The dialysis was carried out for 24 hr at 37°C.

Sample analysis

Misonidazole and desmethylmisonidazole were measured in plasma, CSF and urine extracts by a modification of the gas chromatographic method described by [9]. The compounds were analyzed as their silylated derivatives using a Model 5710 gas chromatograph equipped with a nitrogen phosphorous detector, Hewlett Packard Co., Palo Alto, Calif. Separation was achieved with a 2 × 1.2 m glass column packed with 2% OV-101 on 100/120 mesh Chromosorb W-HP. Helium (flow rate 30 ml/min) was used as the carrier gas. The column temperature was 170°C, and the injector and detector were held at 250 and 300°C, respectively. Plasma, CSF, and urine samples were treated as follows: aliquots (0.1–0.2 ml) were placed in 13 ml glass stoppered centrifuge tubes and were buffered to pH 10 with a 0.1 M solution of Na_2CO_3 (0.5 ml). The solution was extracted with 5 ml of ethylacetate containing the internal standard (2 μg/ml). The ethylacetate was transferred to another 13 ml glass stoppered centrifuge tube and evaporated to dry-

ness at room temperature under a stream of dry N_2. The silyl derivatives were formed by adding 100 μl of BSTFA and 150 μl of acetonitrile to the residue and reacting at room temperature for 15 min. Excess reagent was removed under a stream of dry N_2 at room temperature. The residue was dissolved in 200 μl of benzene and 1 μl of the final solution injected into the gas chromatograph for analysis. Figure 1 shows a typical gas chromatographic tracing of a monkey's plasma extract containing the internal standard, misonidazole and desmethylmisonidazole. Quantitation was achieved by comparing the peak heights of the drug and metabolite to the peak height of the internal standard. The analysis was linear over the range 5 ng–1 μg of misonidazole or desmethylmisonidazole injected on column.

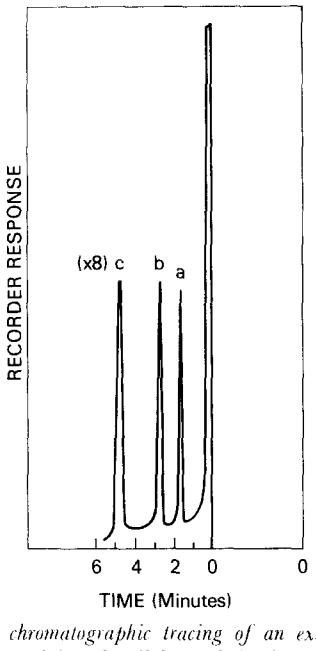

Fig. 1. Gas chromatographic tracing of an extract from monkey plasma containing the silylated derivatives: (a) *the internal standard* (50 ng *injected*); (b) *misonidazole* (50 ng *injected*); (c) *desmethylmisonidazole* (10 ng *injected*).

Electron impact mass spectral identification of the reference compounds, their silylated derivatives and the silylated biological extracts were obtained with a Hewlett Packard Model 5908A gas chromatograph/mass spectrometer equipped with a 3552 data system, Hewlett Packard Co., Palo Alto, Calif. The gas chromatographic parameters were the same as described for quantitation of the compounds. The transfer line was 250°C, and the ionizing energy was 70 eV.

Pharmacokinetics analysis

Plasma and CSF samples and 24-hr urine collections (experiments 1, 4 and 5) were analyzed for misonidazole and desmethylmisonidazole. Least squares analysis of the data was implemented on the NIH DEC system-10 using the MLAB on line modeling laboratory. Plasma and CSF misonidazole levels following i.v. administration best fit monoexponential and biexponential functions, respectively (Fig. 2). CSF levels of misonidazole measured after i.t. injection of the drug were fit to a biexponential function (Fig. 3).

The exponential functions were used to compute the pharmacokinetic parameters summarized in Table 1. The areas under misonidazole CSF $(AUC)_c$ and plasma $(AUC)_p$ vs time curves were evaluated by integrating the respective exponential functions from time equals zero to infinity. The plasma clearance of misonidazole was obtained by dividing the dose by the $(AUC)_p$. Renal clearance of the drug was estimated from the quotient of the amount of drug appearing in the urine in the 24-hr collection period and the $(AUC)_p$ for the same time interval. The time to reach maximum CSF misonidazole levels was determined by setting the first differential of the CSF versus time

Table 1. Summary of pharmacokinetic data after i.v. administration

Exp. No./ Animal ID	Dose (mg/kg)	V_d (ml/kg)	Plasma $t_{\frac{1}{2}}$ (hr)	Plasma AUC (mg/hr/ml)	Plasma clearance (ml/min)	CSF Time to max. level (min)	CSF AUC (mg/hr/ml)	Renal clearance (ml/min)	Dose recovered in 24-hr urine Drug (%)	Dose recovered in 24-hr urine Metabolite (%)
1/A	100	706	3.00	2206	16.3	89.2	2486	0.5	3.7	21.3
2/B	100	631	3.66	3014	12.0	69.4	3085	N.D.	N.D.	N.D.
3/B'*	100	758	5.43	3716	8.4	85.1	3825	N.D.	N.D.	N.D.
4/A	200	708	4.79	7022	10.4	84.7	7708	0.5	4.7	21.9
5/B	200	705	4.84	7127	9.7	109.0	7725	0.6	6.0	11.2

AUC = area under curve; V_d = volume of distribution; N.D. = not done.
*B' is monkey B at time he developed abnormal liver function.

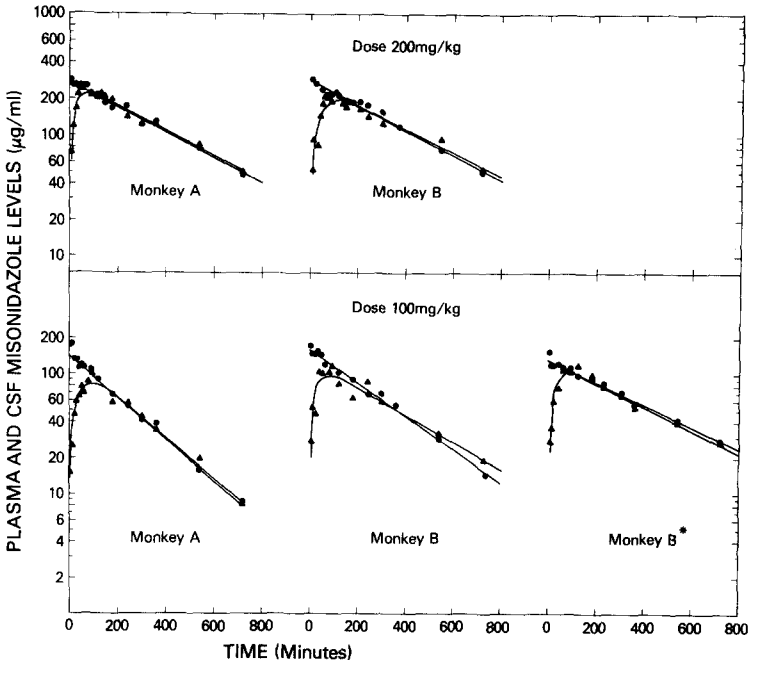

Fig. 2. Plasma and CSF misonidazole levels (μg/ml) *as a function of time over a* 12-hr *period after i.v. administration at* 100 mg/kg (*lower panel*) *and* 200 mg/kg (*upper panel*).

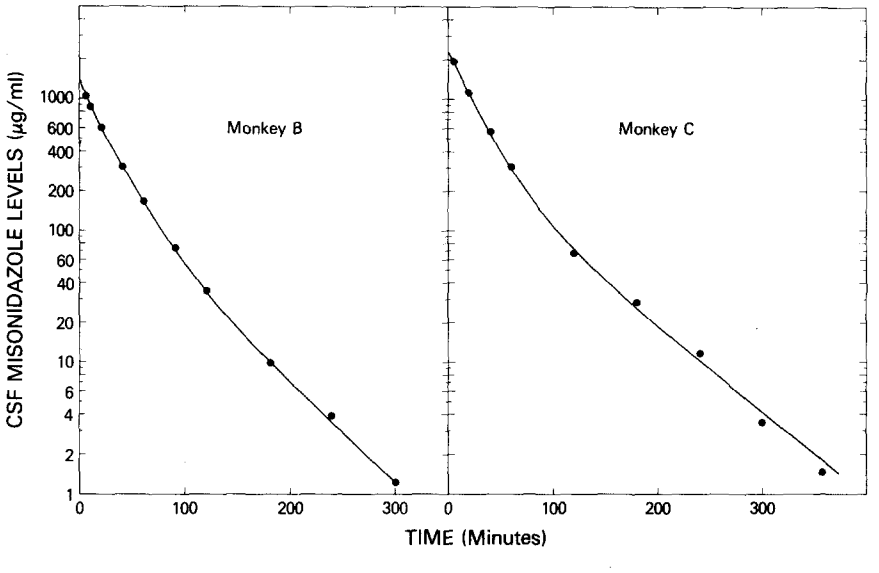

Fig. 3. Misonidazole ventricular CSF levels (μg/ml) *as a function of time following i.t. administration of misonidazole* (0.9 mg/kg).

curve obtained after i.v. administration of misonidazole equal to zero.

RESULTS AND DISCUSSION

Serial CSF and plasma misonidazole levels following i.v. administration are shown in Fig. 2. The same two animals (A and B) were studied at each dose level, 100 and 200 mg/kg. Plasma misonidazole levels decayed in a monoexponential manner. Transfer of drug into the CNS would not be reflected as an additional phase in the plasma level decay curve due to the relatively small volume of the CNS compartment.

Table 1 summarizes the pharmacokinetic data after i.v. administration. The volume of distribution observed was comparable in all experiments, 702 ± 49 mg/kg (mean of 5 experiments), and was similar to total body water in the adult rhesus monkey (686 ml/kg) [10]. Similar distribution volumes have been

observed in humans [11]. Peak plasma levels after 200 mg/kg were double those achieved after 100 mg/kg (300 vs 150 μg/ml). However, the misonidazole plasma half-life appeared to be dose dependent. After the 100 mg/kg dose (experiment 1 and 2), the mean $t_{\frac{1}{2}}$ was 3.3 hr compared to a mean $t_{\frac{1}{2}}$ of 4.8 hr after a 200 mg/kg dose (experiment 4 and 5). The renal and plasma clearance of misonidazole were also evaluated following i.v. administration. Analysis of the 24-hr urine collections resulted in an average recovery of 4.8 and 19.3% of the dose as the parent compound and the desmethylated metabolite, respectively. This low recovery of drug and its metabolite in urine has been reported in several species [9]. These observations suggest that renal excretion of the parent compound is not a major route of drug elimination. As noted above, a comparison of the pharmacokinetic data for the two dose levels showed that doubling the dose increased the plasma half-life. This dose increase produced a 2.7-fold increase in the $(AUC)_p$. This non-linear increase in the plasma $(AUC)_p$ appears to have resulted from a decreased plasma clearance and not from a change in the renal clearance as listed in Table 1. Since metabolism appears to be the major pathway for drug elimination, the difference in the plasma clearance of misonidazole for the two doses suggests the possible saturation of liver microsomal metabolic enzymes. In this regard it was also of interest that a decreased plasma clearance was observed in experiment 3 (Table 1), in which an animal was administered a 100 mg/kg dose at a time when he had developed evidence of impaired liver function i.e., increased SGOT, SGPT, alkaline phosphatase and LDH.

Desmethylmisonidazole levels were also followed after i.v. administration of misonidazole. They were detectable 5 min after administration and steadily rose to approximately 40% of misonidazole plasma levels 4.5 hr later.

CSF levels of misonidazole following i.v. administration, in all five experiments, equilibrated with plasma levels at 88 ± 14 min following i.v. administration. A mean $t_{\frac{1}{2}}$ of 22 ± 5.5 min was calculated for the transfer of the drug from blood into the CSF (see Fig. 2 and Table 1). After equilibrium was achieved, CSF levels decayed in a monoexponential manner at the same rate as the plasma levels. These observations indicate that misonidazole rapidly diffuses across the blood–brain barrier as might be expected for nonionized, lipophilic molecules, which are not protein bound [12]. Misonidazole protein binding was found to be less than 3% bound to serum albumin. In addition, misonidazole has a relatively high octanol/water partition coefficient [13].

Desmethylmisonidazole levels were measured in the CSF and reached 33–82% of those in the plasma 6 hr after misonidazole administration. These observations suggest that desmethylmisonidazole may penetrate the CNS to a lesser extent than the parent compound.

There was no toxicity observed after the i.v. studies over a 3-month observation period; in particular no nausea or vomiting was encountered with this route of administration.

Peak CSF misonidazole levels measured after an i.t. injection of 0.9 mg/kg were greater than 1000 μg/ml (Fig. 3). Despite the high levels achieved no acute or delayed toxicity was observed. This absence of toxicity suggests that CNS effects, i.e., seizures reported in patients, may be related to exposure time rather than to the initial concentration of drug achieved in the CNS.

Figure 3 illustrates the disappearance of misonidazole from the CSF after i.t. administration of 0.9 mg/kg to two monkeys. CSF drug levels decayed in a biexponential manner with average half-times of 15 and 38 min for the fast and slow phases respectively. Volume of distribution for the slow phase averaged 3.3 ml and may be related to the Ommaya reservoir volume plus the ventricular fluid volume. Any detailed suggestions as to the mechanism of transport of misonidazole from the CSF to blood and/or brain would be highly speculative. However, the rapid clearance from the CSF is indicative that CSF bulk flow is of minor importance as a route for the drug elimination. In addition, based on its physical properties (low mol. wt, lipid solubility and non-ionized state), low protein binding and its rapid clearance, it is likely that this drug has a high transcapillary membrane transport as defined by Blasberg *et al.* [14].

The observation that high CSF misonidazole levels were achieved after i.t. administration with a minimal total drug dosage suggests this route of administration as a possible alternative. Since maximum misonidazole tumor levels are required only at the time of radiation therapy the i.t. route might offer the advantage of minimizing the total body tissue exposure to the drug. However, it has suggested that a rapid loss of drug from extracellular fluid by capillary exchange

would reduce the amount present in the extracellular fluid and could significantly retard its diffusional transport into the hypoxic regions of brain tumor tissue [14]. Since the relative rates of diffusion of misonidazole into brain tissue vs transcapillary loss to the systemic circulation after i.t. administration are unknown, it is likely that i.v. rather than i.t. or intra-arterial administration of misonidazole would be the preferred route to obtain high levels of drug in the brain tissue.

The present study demonstrates that i.t. administration of misonidazole results in a very rapid clearance of drug from the CSF and that after i.v. administration, misonidazole levels in the CSF rapidly equilibrate with plasma levels and decay at a similar rate. The pharmacokinetic information presented could prove helpful in establishing the proper timing of radiation in brain tumor patients who have been administered misonidazole as an adjunct to their radiation therapy.

REFERENCES

1. J. F. Fowler, G. E. Adams and J. Denekamp, Radiosensitizers of hypoxic cells in solid tumors. *Cancer Treat. Rev.* **3,** 227 (1976).
2. R. Urtasun, P. Band, J. D. Chapman, M. L. Feldstein, B. Mielke and C. Fryer, Radiation and high dose metronidazole in supratentorial glioblastomas. *New Engl. J. Med.* **294,** 1364 (1976).
3. T. L. Phillips, T. H. Wasserman, R. J. Johnson, C. J. Gomer, G. A. Lawrence, M. L. Levine, W. Sadee, J. S. Penta and D. J. Rubin, The hypoxic cell sensitizer program in the United States. *Brit. J. Cancer*, **37**, (suppl. III), 270 (1978).
4. S. Dische, M. I. Saunders, M. E. Lee, G. E. Adams and I. R. Flockhart, Clinical testing of the radiosensitizer R0070582 experience with multiple doses. *Brit. J. Cancer* **35,** 567 (1977).
5. S. Dische, M. I. Saunders, P. Anderson, R. C. Urtasun, N. Bleethen, K. H. Karcher, H. D. Kogelnix, T. L. Phillips and T. H. Wasserman, The neurotoxicity of misonidazole: pooling of data from five centers. *Brit. J. Radiol.* **51,** 1023 (1978).
6. J. H. Wood, D. G. Poplack, W. J. Flor, E. N. Gumby and A. K. Ommaya, Chronic ventricular cerebrospinal fluid sampling, drug injections and pressure using subcutaneous reservoirs in monkeys. *Neurosurgery* **1,** 2 (1977).
7. J. H. Wood, D. G. Poplack, W. A. Bleyer and A. K. Ommaya, Primate model for long-term study of intraventricularly or intrathecally administered drugs and intracranial pressure. *Science* **195,** 499 (1977).
8. D. G. Poplack, W. A. Bleyer, M. Kostolick, J. L. Savitch and A. K. Ommaya, A primate model for study of methotrexate pharmacokinetics in the central nervous system. *Cancer Res.* **37,** 1982 (1977).
9. I. R. Flockhart, P. Large, D. Troup, S. L. Malcolm and T. R. Marten, Pharmacokinetic and metabolic studies of the hypoxic cell radiosensitizer misonidazole. *Xenobiotica* **8,** 97 (1978).
10. G. H. Bourne, *The Rhesus Monkey: Anatomy and Physiology.* Vol. 1, p. 61. Academic Press, New York (1975).
11. J. M. Strong, J. Schwade, D. G. Poplack and E. Miller, Pharmacokinetic studies of parenteral misonidazole in monkeys and in man. *Proc. Amer. Ass. Cancer Res.* **809,** 200 (1979).
12. D. P. Rall and G. Zubrod, Mechanisms of drug absorption and excretion: passage of drugs in and out of the central nervous system. *Ann. Rev. Pharmacol.* **2,** 109 (1962).
13. P. Wardman, E. D. Clarke, I. R. Flockhart and R. G. Wallace, The rationale for the development of improved hypoxic cell radiosensitizers, *Brit. J. Cancer* **37,** (suppl. III) 1 (1978).
14. R. G. Blasberg, Pharmacodynamics and the blood–brain barrier. Modern concepts in brain tumor therapy. *Nat. Cancer Inst. Monogr.* **46,** 19 (1977).