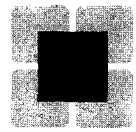

BEN H. BROUHARD, MD, EDITOR

Intra-arterial chemotherapy for brain tumors

SAMUEL J. HASSENBUSCH, MD, PHD; JAMES H. ANDERSON, PHD; DONALD M. WHITING, MD

■ Direct comparisons of theoretical modeling with actual drug delivery can lead to improved brain tumor therapy. In this study, normal and brain tumor-bearing rabbits received infusions of BCNU, or carmustine (1,3-bis [2-chlorethyl]-1-nitrosourea), with ethanol or hyperoxygenated perfluorocarbons as BCNU diluent. When ethanol was used as a diluent, right (infused) hemisphere:left (noninfused) hemisphere ratios of BCNU concentrations in both rabbit groups were markedly lower than had been predicted with theoretical pharmacokinetic modeling. When perfluorocarbons were used as a diluent, ratios of BCNU were significantly improved. These laboratory studies were directly translated into a two-phase protocol for human brain tumor patients. This combined research program demonstrates the successful integration of laboratory and clinical programs.

□ INDEX TERMS: BRAIN NEOPLASMS; CARMUSTINE □ CLEVE CLIN J MED 1990; 57:513–520

THE OBJECTIVE of any laboratory research, especially in neuro-oncology, is improved clinical therapies. Unfortunately, it is often difficult to translate even extensive laboratory studies to significant clinical neuro-oncology programs. The aim of the neuro-oncology research program at the Cleveland Clinic, and the purpose of this report, is to demonstrate the correlation between animal research (both theoretical predictions and actual measurements) and improved human clinical protocols.

The incidence of human brain tumor is high,[1] and despite aggressive therapy involving surgery, radiotherapy, and intravenous (IV) chemotherapy, the median survival rate is only 51 weeks.[2] Intra-arterial (IA) chemotherapy infusions are among the promising new therapies because, in theory, they increase the concentration gradient of the drug from the vascular space to the tumor tissue compartment.[3]

Pharmacokinetic models have been developed to predict and quantify the increased delivery of chemotherapeutic agents to the brain with IA infusions compared to IV infusions.[3,4] These models predict an IA/IV delivery ratio called the IA advantage (R_d), which represents the summation of IA/IV ratios at all time points from the start of the infusion to infinity. The R_d is comparable to the ratio of total drug delivery to infused and noninfused hemispheres after intracarotid infusions.

Few studies have measured drug concentrations in normal or neoplastic brain tissue, and even fewer, if any—whether laboratory or clinical studies—have directly compared pharmacokinetic model predictions with actual experimental drug concentrations. One

From the Department of Neurosurgery, The Cleveland Clinic Foundation (S.J.H., D.M.W.), and the Department of Radiology, The Johns Hopkins Hospital, Baltimore, Maryland (J.H.A.).

Address reprint requests to S.J.H., Department of Neurosurgery, S80-803, The Cleveland Clinic Foundation, One Clinic Center, 9500 Euclid Avenue, Cleveland, Ohio 44195-5226.

human study measured (by positron emission tomography) the pharmacokinetics of the internal carotid artery infusion of BCNU, or carmustine (1,3-bis [2-chlorethyl]-1-nitrosourea). Its results suggested that ^{11}C-BCNU can accumulate to higher concentrations in tumor areas of blood-brain barrier breakdown than in normal brain.[5,6] Direct comparisons to theoretical predictions, however, were not possible in that study.

Chemotherapy in the 1950s and 1960s was delivered by the intracarotid route. Although many drugs were studied, the patient numbers were small and the results inconclusive. Since 1979, many clinical studies have shown improved response rates with IA therapy (especially BCNU) in brain tumor patients.[7–11] Other reports, however, have suggested a lack of clinically beneficial results,[12,13] and even significant toxicity.[7,14,15]

Reported complications of IA chemotherapy with BCNU for brain tumors include acute ocular and neurologic toxicity[16,17] and "leukoencephalopathy" (patchy areas of destruction in the white matter of the cerebral hemispheres).[10,18] When studied in autopsy specimens, these leukoencephalopathic changes consist of perivascular hemorrhage, endothelial atypia, and fibrinoid vascular necrosis; they strongly suggest a vasculopathy as the primary event.[7,15]

ANIMAL STUDIES: EXPERIMENTAL DESIGN

Theoretical modeling predictions

To provide data for theoretical modeling predictions, bilateral carotid blood flows (using ultrasonic flow meters) and BCNU systemic clearances were measured in normal rabbits. Seven normal rabbits were studied to determine values for BCNU systemic clearance. After BCNU was given (300 mg/m^2 as an IV bolus), blood samples were collected over 60 minutes and assayed by a BCNU colorimetric method. A systemic clearance for each rabbit was calculated by dividing the delivered dose (mg/m^2) by the mean blood concentration curve's area-under-curve (AUC). Using the carotid flow rates and systemic clearance values, a theoretical intra-arterial advantage, or R_d, was calculated using the following equation:

$$1 + \frac{\text{systemic clearance}}{\text{carotid flow rate}}$$

where any clearance of the drug by the target organ (the brain in this case) is assumed to be negligible.[4]

Rabbit brain concentrations of BCNU

For the laboratory (preclinical) studies, brain concentrations of BCNU during intra-arterial, or carotid, infusions were determined in three phases. In Phase I, 35 normal (non–tumor-bearing) rabbits received BCNU infusions using the conventional ethanol diluent (BCNU-ethanol) to dissolve the BCNU powder. In Phase II, 25 brain tumor-bearing (VX-2 carcinoma) rabbits received BCNU-ethanol infusions. In Phase III, 8 normal rabbits received infusions using hyperoxygenated perfluorocarbons (BCNU-perfluorocarbon) to dissolve the powder.

Rabbits were sacrificed by pentobarbital overdose at 5, 10, and 15 minutes during the constant infusions, and brains were studied for actual BCNU concentrations at various sites in anterior right and left hemispheres. The data were analyzed as actual concentrations and as matched mirror-image biopsies in the right and left hemispheres. The matched biopsy data were converted into right:left hemisphere concentration ratios. Ratios in the IA-infused rabbits were calculated to provide comparisons of the IA-infused right hemisphere with the noninfused left hemisphere.

Concentrations in the left hemisphere came only from drug passing through the right hemisphere, mixing with the systemic blood in the heart, and then returning to the left hemisphere via the left carotid artery. The left hemisphere concentrations, therefore, represented the equivalent of an IV infusion. The right:left ratio thus was comparable to an IA:IV infusion ratio or an experimental IA advantage at each time point.

The actual concentrations and concentration ratios were then compared with the theoretical predictions and with the other treatment groups.

ANIMAL STUDIES: DESCRIPTION OF TECHNIQUES

All animal experiments were performed on outbred adult New Zealand White rabbits weighing 4 kg to 5 kg. Rabbits were chosen for study because they are relatively inexpensive, their arterial anatomy and the blood-brain barrier in nontumorous areas accurately imitates the same parameters in humans, and their carotid and femoral arteries are large enough to catheterize without significant obstruction.[19]

The VX-2 brain tumor model was used because it results in a reproducible tumor in the brain that alters the blood-brain barrier in tumorous areas in a manner similar to that in the same areas in humans. VX-2 tumor cells transplanted into the rabbit brain produce a solid tumor that has a reliable and reproducible rate of growth. The tumor is approximately 1 cm in diameter and results in death of the animal 12 to 16 days following tumor cell implantation.[20]

Vessel catheterizations and infusions

The carotid artery catheterization, the BCNU dose (20 mg/m²/min to a maximum of 15 minutes), and the 4% ethanol concentration in the normal saline BCNU infusate (in Phase I and II studies) were similar to those used in humans. In all rabbits, a 3.0 French (1.0-mm outer diameter) catheter was passed, using radiographic fluoroscopy, from the right, surgically exposed femoral artery to the right carotid artery and positioned at the level of the third cervical vertebral body.

BCNU was obtained as a powder from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. Perfluorocarbon compound was Adamantine (Adamantech Corporation, Philadelphia, Pa). Perfluorocarbon solutions were supersaturated with oxygen to a P_{O_2} > 600 mmHg before being mixed with BCNU.

Brain samples were obtained from punch biopsies of the frozen brain sections after sacrifice of the animal. BCNU concentrations were assayed in blood samples using a colorimetric assay[21] technique and in brain samples using a modification of a previous gas chromatographic assay.[22]

Statistical evaluations

The data were evaluated by an analysis of variance (General Linear Model [GLM]) procedure using SAS system software (SAS Institute, Inc., Cary, NC) on an IBM PC/AT. All values are expressed as mean ±1 SEM.

RESULTS OF ANIMAL STUDIES

Theoretical modeling predictions

The mean flow in carotid arteries in 32 rabbits was 63.9 ± 3.4 ml/min (237 ml/min/m²). The delivered dose (300 mg/m², average 81 mg) divided by the mean clearance curve's AUC (0.386 min-mg/ml, obtained by trapezoidal method) yielded a systemic clearance of 210 ± 20.6 ml/min (average 778 ± 76.4 ml/min/m²) for these rabbits.

Using the R_d equation shown above and the data for carotid flow and BCNU systemic clearance, an R_d of 4.3 was predicted for total brain exposure to the drug in this system of BCNU infusions. That is, in this animal model, the theoretical prediction is that 4.3 times more drug is exposed to the brain with an IA infusion than with an IV infusion if the same total drug dose is used.

BCNU concentrations

Phase I: Normal rabbits receiving BCNU-ethanol. Average BCNU concentrations in rabbit anterior brains after IA infusions were partially as expected (*Figure 1*). Concentrations were higher in the infusion side, or right hemisphere, than in the left hemisphere. However, the concentrations in the right hemisphere of IA rabbits decreased from 20.5 ± 3.3 μg/g at 5 minutes to 16.4 ± 1.6 μg/g at 10 minutes during the constant infusions. This decrease was not statistically significant.

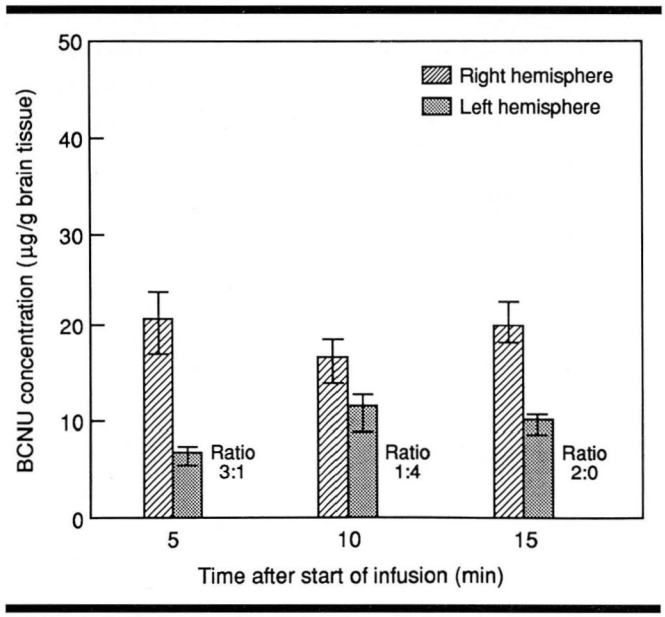

FIGURE 1. BCNU in ethanol infusions in 35 normal, non–tumor-bearing rabbits (20 mg/min/m²). Experimental BCNU concentrations in right (infused) and left (noninfused) brain hemispheres are shown at 5, 10, and 15 minutes after start of right carotid artery constant infusions. Intra-arterial delivery resulted in concentrations in the right hemisphere that decreased from 5 to 10 minutes during the constant infusions. Right:left ratios were lower than theoretically predicted and actually decreased from 5 to 10 minutes ($P < 0.05$).

In the IA animals, the IA advantage ratio at 5 minutes was significantly different from that at 10 minutes ($P < 0.05$, *Figure 1*). Based on the predictions of theoretical modeling, the experimental infused:noninfused (right:left) hemisphere ratios at 5, 10, and 15 minutes during these constant infusions should be significantly higher than the overall theoretical R_d (in this case, 4.3). However, these ratios were all significantly lower than 4.3 and they decreased during the constant infusions.

Phase II: Brain tumor-bearing rabbits receiving BCNU-ethanol. Figure 2 shows the average brain concentrations for tumor biopsies at 5, 10, and 15 minutes after the start of the right IA infusions. In each rabbit, data on the

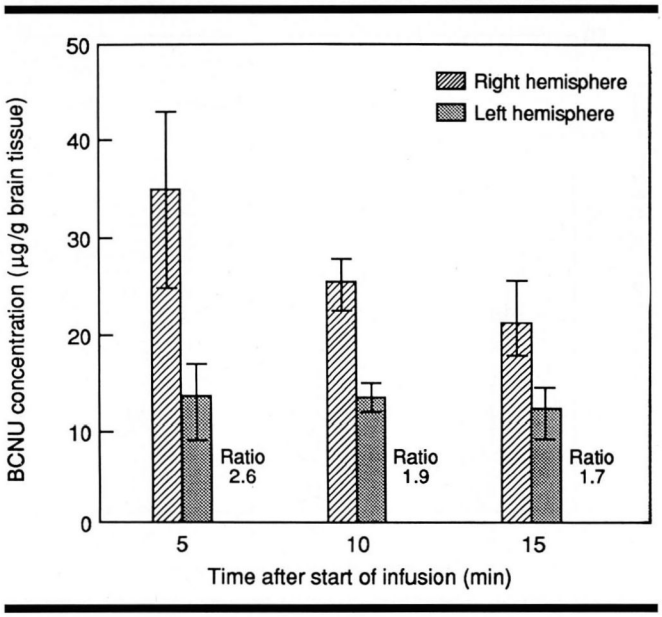

FIGURE 2. BCNU in ethanol infusions in 25 brain tumor-bearing rabbits (20 mg/min/m^2). Experimental brain tumor BCNU concentrations in right (infused) and left (noninfused) brain hemispheres at 5, 10, and 15 minutes after start of right carotid artery constant infusions are shown. The brain tumor was always in the right hemisphere; left hemisphere concentrations represent normal, nontumorous brain. Intra-arterial delivery resulted in infused hemisphere concentrations that were only slightly higher (especially at 10 and 15 minutes) than in the noninfused hemisphere. Concentrations in the infused hemisphere actually decreased during the constant infusions. Right:left ratios were significantly lower than R_d and actually decreased from 5 to 10 minutes ($P < 0.05$).

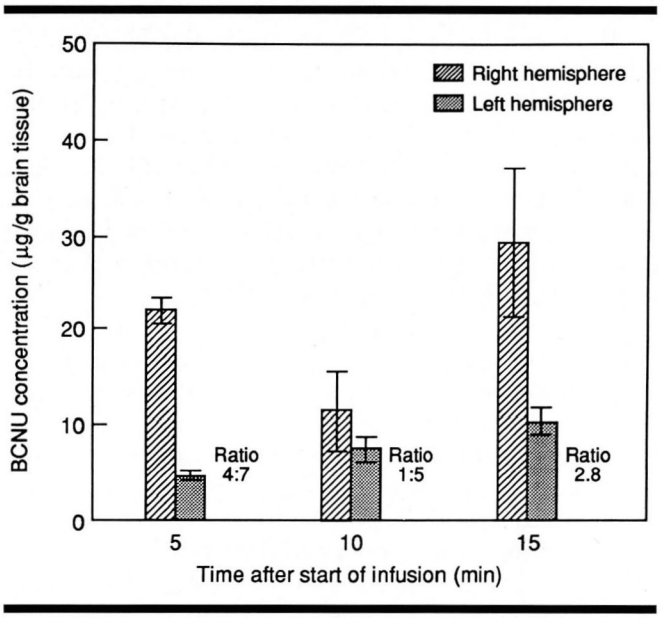

FIGURE 3. BCNU in perfluorocarbon infusions in normal, non–tumor-bearing rabbits (20 mg/min/m^2). Experimental BCNU concentrations in right (infused) and left (noninfused) brain hemispheres at 5, 10, and 15 minutes after start of right carotid artery constant infusions are shown. Right:left ratios were higher at 5 and 15 minutes than corresponding ratios in normal rabbits when ethanol was used as diluent in normal rabbits.

brain concentrations of BCNU in the tumor center and tumor edge were combined because BCNU concentrations from the two areas showed no statistically significant differences. (The tumor was always in the right hemisphere. The left hemisphere values in *Figure 2* relate to biopsies from left hemisphere mirror-image sites corresponding to tumor sites in the right hemisphere.)

The brain concentrations in the right hemisphere (*Figure 2*) also appeared to decrease as the right IA infusion progressed. Right:left concentration ratios at 5 minutes were significantly higher ($P < 0.05$) than those at 10 minutes (*Figure 2*).

Phase III: Normal rabbits receiving BCNU-perfluorocarbon. In normal rabbits, the BCNU concentrations in the brain were higher in the right hemisphere than in the left hemisphere at 5, 10, and 15 minutes (*Figure 3*). Right:left concentration ratios at 5 and 15 minutes were higher than the corresponding experimental ratios for normal rabbits that received BCNU-ethanol.

Perfluorocarbon diluent compared with ethanol. With perfluorocarbon diluent, systemic blood concentrations of BCNU were 22%, 17%, and 13% higher at 5, 10, and 15 minutes, respectively, than with ethanol diluent. The higher blood concentrations of BCNU with perfluorocarbons indicated a slower BCNU clearance in this group at these relatively early time points. The ratios for normal rabbits receiving BCNU-ethanol or BCNU-perfluorocarbon were adjusted for these differences (*Figure 4*). The adjusted values at each time point are expressed as percentages of the ratio predicted by theoretical modeling. Using these adjusted values, at 5 minutes during the constant infusion, the BCNU-perfluorocarbon rabbits achieved ratios statistically closer ($P < 0.05$) to the theoretical predictions for this group than did the BCNU-ethanol rabbits.

CLINICAL PROTOCOLS

These laboratory research results have been used to develop a two-phase human clinical protocol. The

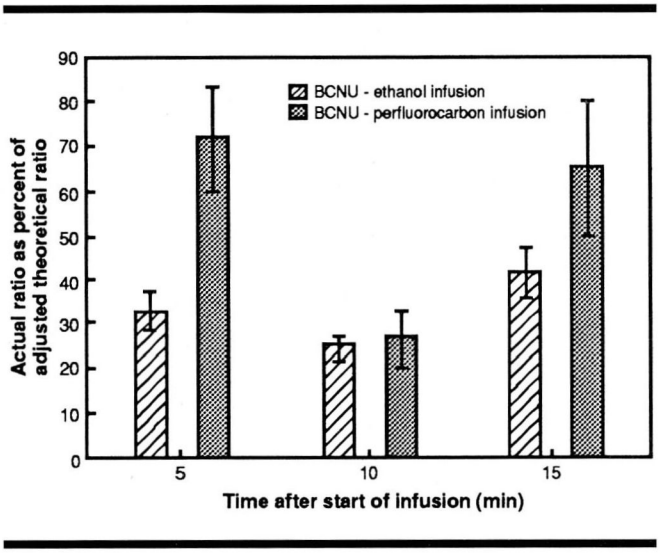

FIGURE 4. BCNU in ethanol and in perfluorocarbon infusions in 43 normal (non–tumor-bearing) rabbits. Right:left hemisphere concentration ratios have been adjusted for differences in systemic BCNU clearance rates and expressed as percentages of the theoretical modeling predictions for the given time point. Percentage of theoretical ratio was significantly higher at 5 minutes ($P < 0.05$) with perfluorocarbon than with ethanol.

protocol uses the same methods for theoretical predictions and for assay of BCNU in brain tissue as do the animal studies, and has been approved by The Cleveland Clinic Foundation Institutional Review Board. The Foundation is accruing patients for Phase I, which is in progress.

In Phase I, primary brain tumor patients (anaplastic astrocytoma or glioblastoma) receive intra-arterial carotid infusions of BCNU-ethanol (200 mg/m^2). Transfemoral catheterization allows placement of the catheter tip above the ophthalmic artery.

For theoretical predictions, blood samples are obtained from patients 1 hour after the start of the 2-hour infusion. Because brain and blood concentrations of BCNU reach steady state during the infusion period, the theoretical modeling is simplified.

Infused carotid artery flow rates are determined in each patient, using Doppler studies to calculate flow velocity and vessel lumen diameter. Samples are obtained by needle biopsy from the edge and the center of the tumor 1 hour after the start of the 2-hour steady state infusions. The use of a computer-guided stereotactic device and computed tomography (CT) enhances visualization and helps to identify the sampling site in the infused hemisphere. The biopsy samples are then assayed for BCNU as in the rabbit studies, using a modification of a gas chromatographic assay.

Systemic blood (venous) concentrations of BCNU are also measured.

BCNU systemic clearance (in ml/min) is calculated by dividing the constant infusion dose rate (in μg/min) by the systemic blood concentration of BCNU (in kg/mL). The R_d for each patient is calculated from the equation presented above.

Under steady-state conditions, the theoretical R_d in each patient should be exactly equal to the BCNU concentration in the infused hemisphere divided by the concentration in the noninfused hemisphere. Biopsy opportunities for the noninfused hemisphere are limited in humans, but the BCNU concentration there can be closely approximated by measuring systemic blood concentrations of BCNU. The noninfused hemisphere is exposed to BCNU only from systemic blood; the high tissue:blood partition coefficient of BCNU results in brain tissue concentrations that are similar to blood concentrations in the same hemisphere.[23] Actual tumor and systemic blood concentrations of BCNU therefore correlate with theoretical modeling predictions, or R_d, and interpatient variability can be determined.

Each patient in Phase I undergoes three courses of intra-arterial infusions, given at 6-week intervals. The therapeutic efficacy of this procedure is measured with serial CT scans for tumor cross-sectional diameter, DNA cell content determined by flow cytometry, and 5-bromodeoxyuridine (BUDR) labeling in brain biopsy samples. BUDR labeling measures the rate of incorporation of IV-administered BUDR (a thymidine analog) into DNA of cells in biopsy specimens examined with light microscopy. The degree of incorporation reflects the proliferative activity of the cells and can indicate the tumoricidal effects of chemotherapy.

In Phase II, patients with primary brain tumor will undergo intra-arterial infusions with BCNU in hyperoxygenated perfluorocarbon. The tumor concentrations of BCNU will be compared with those of BCNU-ethanol infusions from Phase I. As in the Phase I studies, actual brain concentrations will be compared to those predicted by theoretical modeling. Accrual to this protocol will begin within the next year.

DISCUSSION

IA infusions and pharmacokinetic modeling

Therapeutic selectivity is the single most important justification for using intra-arterial chemotherapy in the

cancer patient. Substantial experimental evidence, both in vivo and in vitro, has shown that most tumors have steep chemotherapy dose-response curves; ie, the higher the dose of drug that contacts the tumor, the better the response.[24]

As the IA advantage equation indicates, the R_d is maximized when a drug that has a short systemic half life or high total systemic clearance is delivered into an artery that has a low flow rate. For example, when a drug such as BCNU, with a systemic clearance of approximately 2,040 cc/min,[25,26] is delivered into an artery such as the internal carotid artery, whose flow rate is 480 cc/min,[27] the predicted R_d would be 5.25; ie, the exposure of brain tumor to the same dose of BCNU is 5.25 times greater with IA delivery than with IV delivery.

Limited animal studies of IA therapy[28,29] that compare actual brain concentrations with theoretical modeling predictions have shown that actual concentrations are less than predicted, especially when corrected for actual arterial flow rates as Welch and associates[30] did for the data of Levin and colleagues.[29] Other studies[5,31–33] have measured brain concentrations or the effects of therapy or both, but without direct comparison to the theoretical predictions. In general, these studies have shown only mildly increased drug concentrations and effects with IA as compared with IV infusions.

Theoretical modeling predictions

Using the experimentally determined BCNU systemic clearance and the mean carotid artery flow rate in the study in rabbits, a theoretical intra-arterial advantage, or R_d, of 4.3 was predicted. The theoretical ratio is similar to the 5.25 ratio predicted for humans.

Because the derivation of the R_d formula depends on partial differential equations, it is difficult to predict the ratio at any specific time point during the infusion. At very late time points, however, the experimental ratio of concentrations in the infused and noninfused hemispheres during IA infusions will theoretically approach the R_d ratio. At time points close to the start of the constant infusion, the ratio of experimental concentrations will approach infinity. The experimentally determined concentration ratio at any time point, therefore, should be somewhere between infinity and the steady state value, and the ratio should be higher at earlier time points.

In the rabbit study, BCNU was infused to a maximum of 15 minutes. BCNU pharmacokinetics in rabbits suggest a terminal compartment half life of 36.5 minutes. The actual ratios at 5, 10, and 15 minutes during the constant infusions should be considered as early time points and should be significantly higher than the R_d value.

BCNU concentrations

Phase I: Normal rabbits receiving BCNU-ethanol. The BCNU concentrations in the right (infused) hemisphere at 5 minutes were only 3 times higher than in the left hemisphere during intra-arterial (IA) infusions (*Figure 1*). The difference should have been greater than the expected overall R_d of 4.3 because higher ratios are expected at time points early in the infusion. This lower-than-expected ratio at 5 minutes, combined with the trend for the actual concentrations to be much lower at 10 minutes during the constant infusions, strongly suggests that toxic processes were occurring during the infusions that limited the movement of BCNU into the brain tissues.

Phase II: Brain tumor-bearing rabbits receiving BCNU-ethanol. The results of brain tumor biopsies, which also showed ratios much lower than expected (*Figure 2*), suggested processes during the infusions that limited the delivery of BCNU to the brain tumor tissue. The nature of these processes remains unclear.

Phase III: Normal rabbits receiving BCNU-perfluorocarbon. Although initially studied for oxygen-carrying capabilities, perfluorocarbons have, more recently, been studied for their emulsion properties, ability to act as carriers for other chemicals and drugs, and potentiation of chemotherapeutic agents.[34,35] Studies with various chemotherapeutic agents have shown benefits in many animal tumor models. The mechanisms appear to be oxygenation of hypoxic tumor cells and, possibly, effects of perfluorocarbon emulsion on drug partitioning in blood and, therefore, presentation of the chemotherapeutic agent to the tumor.[36] Perfluorocarbons also may lessen protein binding of the drug in blood.[37]

The use of perfluorocarbons with IA chemotherapy infusions, however, has not been studied extensively. In our study, the ratios of right:left brain concentrations of BCNU with perfluorocarbons as diluent were significantly increased, especially at 5 minutes, as compared with BCNU-ethanol ratios (*Figure 4*), and more nearly approached the theoretical modeling predictions.

Laboratory research and human protocols

The two-stage clinical protocol described in this report evolved directly from the results of animal studies and offers a new concept and new technique for IA therapy.

In the animal studies, the direct comparison of theoretical predictions with actual brain drug concentrations suggested modifications needed in IA

delivery to improve actual brain concentrations. Because of observations that the experimental concentration ratios of BCNU with ethanol were significantly less than predicted by pharmacokinetic modeling, the clinical protocol was then modified to use the perfluorocarbon diluent.

This extension to humans of the comparison between theoretical modeling and measurement of actual brain concentrations is among the first of such efforts. Continued improvements in this relationship will enhance the understanding of brain tumor therapy and the efficacy of therapy. For example, the use of hyperoxygenated perfluorocarbons in Phase II of this clinical protocol is based directly upon study of the theoretical-actual relationship.

The special technique involved in this translation from laboratory to clinical protocol is the use of stereotactic biopsies to measure actual brain concentrations. The practical availability, common use, and relative safety of CT-guided stereotactic biopsies, coupled with the high-sensitivity gas chromatographic tissue assay for BCNU (specifically developed for this research), have made this advance possible.

Laboratory investigations are now underway into the causes, such as possible local toxicity, of the discrepancy between the theoretical and actual concentrations reported in these studies. These include a study of carotid artery flow rates during infusion and a histologic study of arteriolar and capillary vasculature in the brain parenchyma. Other ongoing laboratory studies with short-term direct clinical application include the examination, in both normal and brain tumor-bearing animals, of the theoretical and experimental concentrations of other chemotherapeutic agents. These new studies will examine the concepts of water solubility and half life (eg, other nitrosourea agents with systemic clearance that is 10 times faster than that of BCNU).

ACKNOWLEDGMENTS

This work was supported by National Cancer Institute Grant Number CA36920 and by a Young Investigator Research Fellowship and a Faculty Research Grant from the American Association of Neurological Surgeons. BCNU drug was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute.

The authors thank Michael Samphilipo, BS, and Frank Starr III, RT (ARRT), for their indispensable work in the experimental design and completion of these studies.

REFERENCES

1. Office of Biometry and Epidemiology, Natl Inst Neuro and Communicative Disorders and Stroke. Survey of Intracranial Neoplasms. Final Report. Bethesda, National Institutes of Health, 1977.
2. Walker MD, Green SB, Byar DP, et al. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med 1980; **303:**1323–1329.
3. Cowles AL, Fenstermacher JD. Theoretical considerations in the chemotherapy of brain tumors [In] Farah A, Welch AD, eds. Handbook of Experimental Pharmacology. New York, Springer-Verlag, 1974, pp 319–329.
4. Collins JM. Pharmacologic rationale for regional drug delivery. J Clin Oncol 1984; **2:**498–504.
5. Diksic M, Sako K, Feindel W, et al. Pharmacokinetics of positron-labeled 1,3-Bis(2-chloroethyl)nitrosourea in human brain tumors using positron emission tomography. Cancer Res 1984; **44:**3120–3124.
6. Yamamoto YL, Diksic M, Sako K, et al. Pharmacokinetic and metabolic studies in human malignant glioma. [In] Magistretti PL, ed. Functional Radionuclide Imaging of the Brain. New York, Raven Press, 1983, pp 327–335.
7. Foo SH, Ransohoff J, Berenstein A, Choy IS. Intra-arterial BCNU chemotherapy for malignant gliomas. J Neurosurg 1985; **62:**458–459.
8. Hochberg FH, Pruitt AA, Davis K, DeBruyn G. Intra-arterial BCNU chemotherapy of glioblastoma. Neurology 1983; **33 [Suppl 2]:**109.
9. West CR, Avellanosa AM, Barua HR, Patel A, Hong CI. Intra-arterial 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and systemic chemotherapy for malignant gliomas. A follow-up study. Neurosurgery 1983; **13:**420–426.
10. Greenberg HS, Ensminger WD, Chandler WF, et al. Intra-arterial BCNU chemotherapy for treatment of malignant gliomas of the central nervous system. J Neurosurg 1984; **61:**423–429.
11. Cascino TL, Byrne TN, Deck MD, Posner JB. Intra-arterial BCNU in the treatment of metastatic brain tumors. J Neurooncol 1983; **1:**211–218.
12. Papavero L, Loew F, Jaksche H. Intracarotid infusion of ACNU and BCNU as adjuvant therapy of malignant gliomas. Clinical aspects and critical considerations. Acta Neurochir 1987; **85:**128–137.
13. Shapiro WR, Green SB. Reevaluating the efficacy of intra-arterial BCNU [letter]. J Neurosurg 1987; **66:**313–315.
14. Kapp JP, Sanford HA. Neurological deficit after carotid infusion of cisplatin and 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) for malignant glioma: an analysis of risk factors. Neurosurgery 1986; **19:**779–783.
15. Kleinschmidt-DeMasters BK. Intracarotid BCNU leukoencephalopathy. Cancer 1986; **57:**1276–1280.
16. Grimson BS, Mahaley MS Jr, Dubey HD, Dudka L. Ophthalmic and central nervous system complications following intracarotid BCNU (Carmustine). J Clin Neuro Ophthalmol 1981; **1:**261–264.
17. Gebarski SS, Greenberg HS, Gabrielsen TO, Vine AK. Orbital angiographic changes after intracarotid BCNU chemotherapy. AJNR 1984; **5:**55–58.
18. Greenberg HS, Ensminger WD, Seeger JF, et al. Intra-arterial BCNU chemotherapy for the treatment of malignant gliomas of the central nervous system: A preliminary report. Cancer Treat Rep 1981; **65:**803–810.
19. Jeppsson PG, Olin T. Cerebral angiography in the rabbit. Lunds Universitets Arsskrift 1960; **56:**1–56.
20. Carson BS, Anderson JH, Grossman SA, et al. Improved rabbit brain tumor model amenable to diagnostic radiographic procedures. Neurosurgery 1982; **11:**603–608.
21. Loo TL, Dion RL. Colorimetric method for the determination of 1,3-Bis(2-chloroethyl)-1-nitrosourea. J Pharm Sci 1965; **54:**809–810.
22. Smith RG, Blackstock SC, Cheung LK, Loo TL. Analysis for nitrosourea antitumor agents by gas chromatography - mass spectrometry. Anal Chem 1981; **53:**1205–1208.
23. Levin VA, Patlak CS, Landahl HD. Heuristic modeling of drug delivery to malignant brain tumors. J Pharmacokinet Biopharm 1980; **8:**257–296.
24. Blum RH, Frei E, Holland JF. Principles of dose, schedule, and combination chemotherapy. [In] Holland JF, Frei E, eds. Cancer Medicine.

Philadelphia, Lea and Febiger, 1982, pp 730–752.
25. Levin VA, Hoffman W, Weinham RJ. Pharmacokinetics of BCNU in man. A Preliminary study of 20 patients. Cancer Treat Rep 1978; **62:**1305–1312.
26. Oldfield EH, Dedrick RL, Yeager RL, et al. Reduced systemic drug exposure to combining intra-arterial chemotherapy with hemoperfusion of regional venous drainage. J Neurosurg 1985; **63:**726–732.
27. Uematsu 5, Yang A, Kouba R. Normal and abnormal common carotid artery blood flow by transcutaneous ultrasonic volume flow meter. [In] Dietrich EG, ed. Non-invasive Assessment of the Cardiovascular System. Littleton, Mass., PSG Inc., 1982, pp 87–96.
28. Egorin MJ, Bellis EH, Salcman M, Collins JM, Spiegel JF. The pharmacology of diaziquone given in intravenous or intracarotid infusion to normal and intracranial tumor-bearing puppies. J Neurosurg 1984; **60:**1005–1013.
29. Levin VA, Kabra PM, Freeman-Dove MA. Pharmacokinetics of intracarotid artery ^{14}C-BCNU in the squirrel monkey. J Neurosurg 1978; **48:**587–593.
30. Welch KMA, Spira PJ, Knowles L, Lance JW. Effects of prostaglandins on the internal and external carotid blood flow in the monkey. Possible relevance to cranial flow changes during migraine headache. Neurology 1974; **24:**705–710.
31. Cohen AR, Peietronigro DD, Cravioto H, Flamm ES. Effect of-difluoromethylornitnine on the antiglioma therapeutic efficacy of intra-arterial BCNU. J Neurosurg 1986; **65:**671–678.
32. Bullard DE, Bigner SH, Bigner DD. Comparison of intravenous versus intracarotid therapy with 1,3-Bis (2-chloroethyl)-1-nitrosourea in a rat brain tumor model. Cancer Res 1985; **45:**5240–5245.
33. Hiesiger EM, Basler GA, Lipschutz L, Shapiro WR. Quantitative autoradiographic (QAR) determination of ^{14}C-PCNU concentration [^{14}C-PCNU] in C6 rat glioma after intracarotid (IC) administration. Proc Amer Assoc Cancer Res 1985; **26:**349.
34. Kuwamura K, Kokunai T, Tamaki N, Matsumoto S. Synergistic effect of perfluorochemicals on BCNU chemotherapy. Experimental study in a 9L rat brain-tumor model. J Neurosurg 1982; **57:**467–471.
35. Teicher BA, Holden SA, Rose CM. Differential enhancement of melphalan cytotoxicity in tumor and normal tissue by Fluosol-DA and oxygen breathing. Int J Cancer 1985; **36:**585–589.
36. Yuhas JM, Goodman RL, Moore RE. Potential application of perfluorochemicals in cancer therapy. Int Anesthesiol Clin 1985; **23:**199–209.
37. Weinkam RJ, Finn A, Levin VA, Kane JP. Lipophilic drugs and lipoproteins: Partitioning effects on chloroethylnitrosourea reaction rates in serum. J Pharmacol Exp Ther 1980; **214:**318–323.