SPECT Quantitation of Cobalt-57 Bleomycin Delivery to Human Brain Tumors

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A newly developed and validated noninvasive quantitative SPECT method was used to measure the in vivo uptake of[^{57}Co]bleomycin (Co-bleo) in 13 human brain tumors and the uptake of [^{99m}Tc]glucoheptonate (GH) in 23 brain tumors. Significant differences in tumor uptake were found. The tumor concentration over time, the tumor to blood radio at 30 min and the tumor cumulative concentration of radioactivity showed marked differences even between tumors with the same histology. Only a weak correlation was found between tumor concentration of Co-bleo and of GH. Therefore a simple imaging agent such as GH cannot, at the present time, serve as an indicator of individual tumor uptake and further experience with other agents is still necessary. Contrary to the generally held view, no correlation was found between the concentration of drug in the blood and its tumor concentration. It is suggested therefore that the level of a drug in the blood cannot be used as a criterion of the amount that will penetrate the tumor. Direct SPECT measurement of the concentration of the drug in the tumor itself should be performed. The bioavailability of a drug is critical in order for it to exert it tumoricidal effect. The results, showing marked differences in uptake between brain tumors, suggest that before chemotherapy is administered, uptake of the chemotherapeutic drug in the individual tumor to be treated should be assessed and comparisons should be made between the uptake of a series of drugs to determine which drug would be most efficacious on the basis of its uptake as well as its tumor cell killing potential.

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Better diagnosis of brain tumors by CT or NMR has not improved disease prognosis. It also appears that improvements in surgical and radiation therapy techniques over the past two decades have not resulted in improvement in patient survival (*1–3*). Chemotherapy which may be effective in tumors outside the central nervous system is in general useless in the treatment of brain tumors. The blood-brain barrier is assumed to be the main factor that limits the use of antineoplastic drugs in brain tumors (*4–7*). Treatment by lipid soluble chemotherapeutic agents which cross the blood-brain barrier has been attempted for a number of years (*8*). However, treatment with these agents also has had only limited success and does not appear to have improved significantly the bleak prognosis of patients with brain tumors.

Recently, however, there has been renewed interest in the treatment of brain tumors. New drugs that do not cross the blood-brain barrier, but are effective in extracerebral tumors, are being tested (*1,9*). The basis for their use is that the blood-tissue barrier of a brain tumor is not identical with the blood-brain barrier of normal brain and therefore brain tumors may be permeable, to some degree, to water soluble drugs (*7*). In addition, experiments are being conducted using techniques which open the blood-brain barrier and blood-tissue barrier to enable better access of such drugs into brain tumors (*10*). This research is, however, limited by the lack of quantitative methods for the measurement of drug uptake by human brain tumors. Estimation of the amount of drug that enters human brain tumors is based mainly on results from animal tumors or on theoretical models (*4,11–13*).

In the present paper we have used a noninvasive in vivo quantitative SPECT technique that has been recently validated (*14*) to characterize the blood-tissue barrier of different human brain tumors. We have determined the uptake of Cobalt-57 (^{57}Co) bleomycin

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(Co-bleo), a labeled chemotherapeutic drug, into human brain tumors and studied, the in vivo pharmacokinetics of this agent in tumors in situ. Uptake of Co-bleo was also compared to that of technetium-99m (^{99m}Tc) glucoheptonate (GH) to determine whether a simple brain scanning agent could serve as an indicator of brain tumor permeability to chemotherapeutic drugs.

MATERIALS AND METHODS

Thirteen patients with newly diagnosed brain tumors had Co-bleo SPECT, and 23 patients had GH SPECT studies of their brain. Nine patients had both studies with Co-bleo and Tc-GH performed. They included four patients with gliomas, four with metastases, and one with a meningioma. All patients had a routine brain tumor neurologic evaluation which included transmission CT. The research protocol for the study was approved by our institution. Cobalt-57 (DuPont Company, No. Billerica, MA) and glucoheptonate were generously donated for this study. Co-bleo was prepared in our laboratory as previously described (*15,16*). Two millicuries of ^{57}Co were injected intravenously for each study and 1 mg of bleomycin was labeled with 1 mCi of ^{57}Co (specific activity 1 mCi/mg); uptake was measured by SPECT as percent of injected dose per cubic centimeter of tissue (%ID/cc) and was recalculated as microgram bleomycin/cc of tumor tissue.

Glucoheptonate labeled with 20–25 mCi of ^{99m}Tc by the routine procedure was injected intravenously. Since the specific activity of glucoheptonate was not available for all patients, concentration of glucoheptonate in the tumor was measured only as %ID/cc. Decay corrections were applied. Nine patients had both Tc-GH and Co-bleo studies. Technetium-99m glucoheptonate always preceded Co-bleo, and the second study was done at least 2 days later when no significant ^{99m}Tc activity was present in the tumor. Blood activity was not corrected for since the blood volume of brain tumors $< 5\%$ of the volume of the tumor (*17*).

We have used a SPECT method developed for in vivo quantitation (*14*). The studies were performed using an Apex-415 digital gamma camera (Elscint, Inc., Haifa, Israel) with a rotating gantry and an all-purpose, low-energy collimator. The data collected was stored on an optical disk (Sudbury Systems, Inc., Sudbury, MA). Data acquisition required 120 projections 3° apart when using ^{99m}Tc and 60 projections 6° apart when using ^{57}Co. The difference in the number of projections required for Tc-GH and Co-bleo studies results from the significant difference in the amount of injected radioactivity. The acquisition time per study was 20 min. The rotation radii for SPECT studies ranged from 17–20 cm. The system resolution is 0.68 cm. The 64 × 64 matrix was used for both radioisotopes. For ^{99m}Tc, 33,000–50,000 counts were collected for each projection, and the entire study accumulated 4–6 × 10^6 counts. When using ^{57}Co, 2,500–4,000 counts were collected for each projection and 1.5–2.4 × 10^5 were accumulated for the entire study. The data was reconstructed by backprojection, using a Hanning filter, in the transverse, sagittal, and coronal planes. The tomographic slice thickness was one pixel (0.68 cm). No smoothing was used. A threshold of 0.43 (43% of the maximal counts in a slice) was used to define the tumor. This threshold was experimentally found to give the best correlation between measured and real volume and concentration both in phantoms and when in vivo and in vitro studies were compared in human brain tumors (*14*). For each slice the number of pixels, containing counts that exceeded that of the threshold, was used to calculate the volume. The number of counts in the volume delineated was calculated by the computer and compared to the amount of actual activity in phantoms in order to obtain the concentration of the radioactive drugs in μCi/cc (*14*). The absolute value of the threshold is variable from study to study according to 43% of maximal counts in the slice. Such a threshold gives an excellent correlation for the range of volumes of brain tumors found both in the literature and our own study.

The same SPECT procedure was used for each tumor at 30, 120, 240, and 480 min after i.v. injection of the labeled drug. Blood samples were taken every 30 sec for 5 min, at 10 and 20 min and at intervals thereafter before each SPECT study. The cells were separated, and plasma activity was measured in a well counter.

The integral of the concentration in the tumor (CT) between 30 min and 480 min was defined as the tumor cumulative concentration (TCC) and was calculated using the following formula:

$$TCC = \int_{30'}^{480'} CT\ (t)dt$$

It is used to represent the amount of the drug to which the tumor was exposed during the period of the study.

For assessing the variability of the TCC in different tumors it was normalized to the integral of blood activity. The tumor cumulative concentration normalized for blood (TCC/B) was calculated as follows:

$$TCC/B = \frac{TCC}{\int_{0}^{480'} CB(t)\ dt}$$

where CB indicates the concentration in the blood.

The tumor to blood ratio (TBR) was determined at 30 min as an indication of tumor permeability. It was calculated using the formula:

$$TBR = \frac{CT\ (30')}{\int_{0}^{30'} CB(t)\ dt}$$

RESULTS

Tumor volume in our patient population ranged between 30 and 120 cc. There was a marked variability in concentration of the administered drug among the tumors studied. This variability was apparent even in tumors with the same histology.

When using Co-bleo in gliomas, the maximal difference in concentration (292%) appeared at 120 min with values of 0.013–0.051 μg/cc; the minimal difference (170%) was found at 240 min with concentrations of

0.010–0.027 μg/cc. In meningiomas maximal difference (127%) was measured at 480 min with values ranging from 0.018–0.041 μg/cc, and the minimal difference (43%) was measured at 120 min with values of 0.035–0.050 μg/cc. In metastases the maximal difference (220%) was found at 30 min with a range of 0.20–0.064 μg/cc, and the minimal difference (25%) appeared at 480 min with values of 0.020–0.025 μg/cc. The TBR varied between 3.9–8.9 × 10^{-3} min^{-1} in gliomas, 7.0–11.2 × 10^{-3} min^{-1} in meningiomas, and

TABLE 1
Concentration of Co-bleo in the Blood and in Brain Tumors in 13 Patients

Histology of tumor	No. pts.	30′	120′	240′	480′	TBR*	TCC†	TCC/B‡
Glioma	B§	6						
	T**	6						
Minimal	B	0.076	0.06	0.024	0.003		12.36	
value	T	0.019	0.013	0.01	0.009	3.9	5.08	0.35
Maximal	B	0.33	0.14	0.066	0.02		34.1	
value	T	0.06	0.051	0.027	0.029	8.9	15.53	0.57
Mean	B	0.165	0.091	0.045	0.01		24.9	
	T	0.037	0.033	0.023	0.021	5.9	11.6	0.47
Standard	B	0.082	0.028	0.013	0.007		7.07	
deviation	T	0.014	0.012	0.006	0.006	1.82	3.21	0.07
Range	B	0.254	0.08	0.042	0.017		21.74	
	T	0.041	0.038	0.017	0.02	4.6	10.45	0.22
Variation	B	50%	31%	28%	68%		28%	
coefficient	T	40%	35%	26%	30%	31%	28%	16%
Meningioma	B	3						
	T	3						
Minimal	B	0.11	0.046	0.014	0.001		11.2	
value	T	0.03	0.035	0.024	0.018	7.0	12.12	0.52
Maximal	B	0.159	0.078	0.047	0.018		23.15	
value	T	0.05	0.05	0.045	0.041	11.2	20.52	1.20
Mean	B	0.127	0.065	0.029	0.007		17.32	
	T	0.041	0.041	0.031	0.027	9.5	14.97	0.96
Standard	B	0.022	0.014	0.014	0.007		4.88	
deviation	T	0.008	0.007	0.01	0.01	1.8	3.93	0.31
Range	B	0.049	0.032	0.033	0.016		11.95	
	T	0.02	0.015	0.021	0.023	4.2	8.41	0.68
Variation	B	18%	21%	48%	100%		28%	
coefficient	T	20%	18%	32%	39%	19%	26%	32%
Metastases	B	4						
	T	4						
Minimal	B	0.098	0.054	0.025	0.005		14.13	
value	T	0.02	0.026	0.023	0.020	3.1	10.3	0.53
Maximal	B	0.15	0.088	0.046	0.013		24.02	
value	T	0.064	0.042	0.034	0.025	14	16.02	1.14
Mean	B	0.124	0.072	0.035	0.008		19.34	
	T	0.04	0.035	0.027	0.023	7.8	12.87	0.73
Standard	B	0.022	0.016	0.01	0.003		4.59	
deviation	T	0.016	0.007	0.004	0.002	2.06	4	0.25
Range	B	0.052	0.034	0.021	0.008		9.89	
	T	0.044	0.016	0.011	0.005	5.72	10.9	0.61
Variation	B	18%	22%	27%	38%		24%	
coefficient	T	40%	20%	15%	8%	51%	16%	34%

* Tumor blood ratio at 30 min (× 10^{-3} min^{-1}).
† Tumor cumulative concentration (μg/cc × min).
‡ Tumor cumulative concentration normalized for blood cumulative concentration.
§ Blood (μg/cc).
** Tumor (μg/cc).

TABLE 2
Concentration of GH in the Blood and in Brain Tumors in Eight Patients with Gliomas and Metastases After a Co-bleo Study*

Histology of tumor	No. pts.	30′	120′	240′	480′	TBR†	TCC‡	TCC/B§
Glioma	B**	3						
	T¶	4						
Minimal	B	3.54	2.37	1.38	0.31		0.684	
value	T	0.61	0.55	0.47	0.54	5.44	0.231	0.34
Maximal	B	8.38	4.85	4.05	2.83		1.827	
value	T	2.06	1.70	1.7	1.93	7.97	0.793	0.53
Mean	B	6.06	3.85	2.41	1.20		1.204	
	T	1.48	1.22	1.09	1.09	6.36	0.517	0.43
Standard	B	1.98	1.07	1.17	1.15		0.472	
deviation	T	0.54	0.42	0.44	0.52	1.14	0.203	0.08
Range	B	4.84	2.48	2.67	2.52		1.143	
	T	1.45	1.15	1.23	1.39	2.53	0.562	0.19
Variation	B	33%	28%	49%	96%		39%	
coefficient	T	36%	35%	41%	48%	18%	39%	20%
Metastases	B	3						
	T	4						
Minimal	B	4.48	1.13	0.18	0.01		0.292	
value	T	1.20	1.14	1.06	0.88	6.35	0.470	0.16
Maximal	B	5.76	4.35	2.99	1.42		1.679	
value	T	2.66	2.16	1.89	2.08	15.6	0.902	1.86
Mean	B	5.12	2.63	1.42	0.55		0.762	
	T	1.88	1.70	1.52	1.42	9.52	0.708	0.85
Standard	B	0.52	1.32	1.17	0.62		0.648	
deviation	T	0.54	0.42	0.38	0.45	4.30	0.184	0.73
Range	B	1.28	3.22	2.81	1.41		1.387	
	T	1.46	1.02	0.83	1.20	9.25	0.432	1.70
Variation	B	10%	50%	82%	113%		85%	
coefficient	T	29%	25%	25%	32%	45%	26%	86%

* One patient with a meningioma had also both a GH and Co-bleo study.
† Tumor blood ratio at 30 min ($\times 10^{-3}$ min^{-1}).
‡ Tumor cumulative concentration (%ID/cc × min).
§ Tumor cumulative concentration normalized for blood cumulative concentration.
** Blood (%ID/cc × 10^{-3}).
¶ Tumor (%ID/cc × 10^{-3}).

3.1–14.0 × 10^{-3} min^{-1} in metastases. The TCC also showed a large variation 5.08–15.53 μg/cc × min in gliomas, 12.12–20.52 μg/cc × min in meningiomas and 10.3–16.02 μg/cc × min in metastases. The TCC/B varied between 0.35–0.57 in gliomas, 0.52–1.20 in meningiomas and 0.53–1.14 in metastases.

Of the nine patients that had both Co-bleo and GH studies, large variations were found when using GH in gliomas and metastases, only one patient had a meningioma. The maximal difference in concentration (257%) was found in gliomas at 480 min with values of 0.54–1.93 × 10^{-3} %ID/cc. The minimal difference (208%) was found at 120 min with values of 0.55–1.7 × 10^{-3} %ID/cc. In metastases, maximal difference (136%) was found at 480 min 0.88–2.08 × 10^{-3} %ID/cc and minimal (89%) at 120 min 1.14–2.16 × 10^{-3} %ID/cc. The TBR was 5.44–7.97 × 10^{-3} min^{-1} in gliomas, and 6.35–15.6 × 10^{-3} min^{-1} in metastases. The TCC varied from 0.231–0.793 %ID/cc × min in gliomas and 0.470–0.902 %ID/cc × min in metastases. The TCC/B varied in gliomas between 0.34–0.53 and in metastases between 0.16–1.86.

The correlation between the concentrations of Co-bleo and GH in the tumors was 0.69. No correlation was found between the level of Co-bleo in the blood and that in the tumor ($r = 0.47$). The same lack of correlation was found for GH ($r = 0.39$). The minimal and maximal values of concentration over time, the standard deviation, and the variability coefficient of the concentration in the tumors are summarized for Co-bleo in Table 1 and for GH in Table 2. The concentration of Co-bleo in the blood and in the tumor is shown

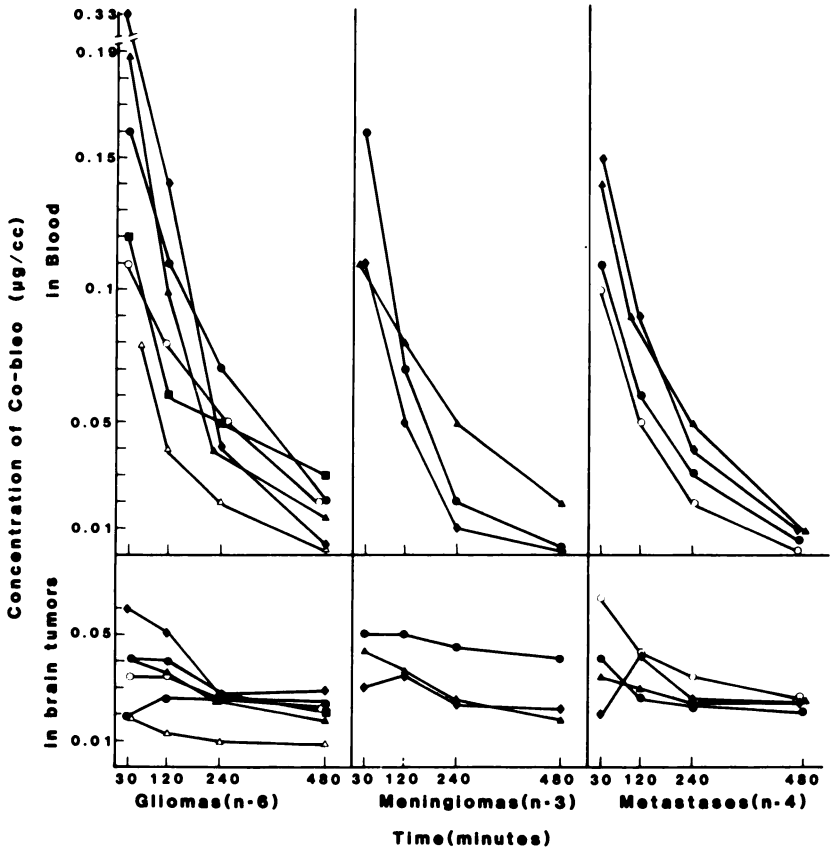

FIGURE 1
Concentration of Co-bleo in 13 patients with brain tumors and in the blood

in Figure 1. The concentration of GH in the blood and in the tumor is shown in Figure 2. The correlation between the concentration of Co-bleo and GH is shown in Figure 3. The correlations between the concentration of Co-bleo and GH in the blood and in the tumors are shown in Figures 4 and 5, respectively.

DISCUSSION

SPECT can be reliably employed to noninvasively measure the concentration of radiopharmaceuticals in brain tumors (*14*). In using SPECT quantitation, an excellent correlation was found between measured and real volumes and concentration in phantoms. When in vivo uptake in patients with meningiomas was compared to the "gold standard" of in vitro measurements in samples of the same tumors taken during operation, a correlation of 0.93–0.84 was found (*14*). The same methods were used in the present study to assess the in vivo pharmacokinetics of Co-bleo and GH in human brain tumors.

SPECT quantitation provides information which can not presently be obtained by any other technique, and the TCC shows the amount of chemotherapeutic drug to which the tumor is exposed—the bioavailability of the drug (*18*). Tumor models in animals are not, in reality, true models of human tumors. In addition, measurement of uptake, although accurate when using the method of quantitative autoradiography (*7,12*), necessitates the sacrifice of the animal and prohibits the measurement of concentration over time in the same tumor. The short half-life of presently used positron emitters would require repeat injections and complicated logistics if PET was to be used for pharmacokinetic studies. Computed tomography and NMR cannot, presently, provide quantitative information about drug uptake.

Cobalt-57 bleomycin was chosen for this study for a number of reasons. Treatment with bleomycin has been attempted in brain tumors, and Co-bleo can be prepared using a simple technique (*15,16*). Furthermore, it has been recently shown that the metal binding capacity of bleomycin is very high both in the extracellular and intracellular space (*19*). The binding does not alter the biodistribution of bleomycin, and it is actually believed to account for its biologic activity. It is the metal bound bleomycin which, presumably, has the tumoricidal effect (*19*). Co-bleo, therefore, can be regarded as representing the behaviour of the nonlabeled bleomycin which is administered to patients for cancer treatment. It does not, however, represent the behaviour of other chemotherapeutic agents.

The results indicate that there is a marked variation in the pharmacokinetics of Co-bleo in human brain tumors. Such a variability occurs even in tumors with the same histology. The variability coefficient showed

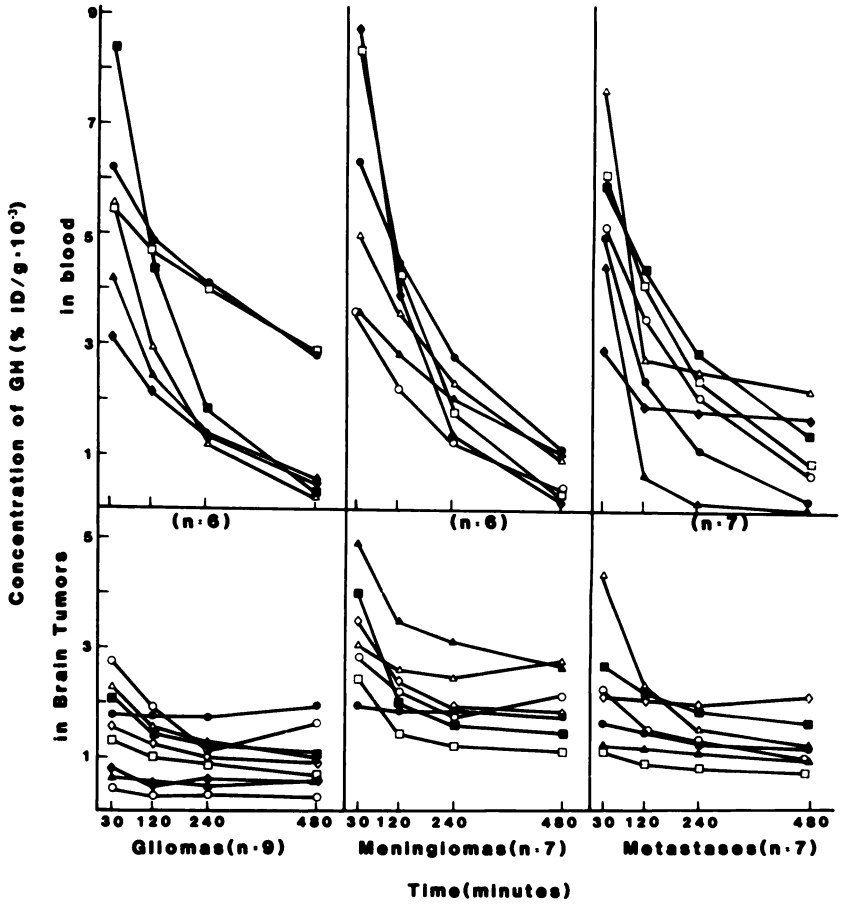

FIGURE 2
Concentration of GH in 23 patients with brain tumors and in the blood

a significant difference (Table 1 and 2) when the concentration of Co-bleo was measured at different times after the i.v. injection, and the same was true for the TBR and the TCC. The results show that human brain tumors may differ markedly in their exposure to drugs and individual tumors, belonging to the same histological group, may have a high or low drug uptake. The dose of drug given to the patient is based on his body surface area and not on the bioavailability of the drug to his individual tumor. Our method will enable, in the future, to tailor the type and amount of drug according to the permeability of the individual tumor at hand.

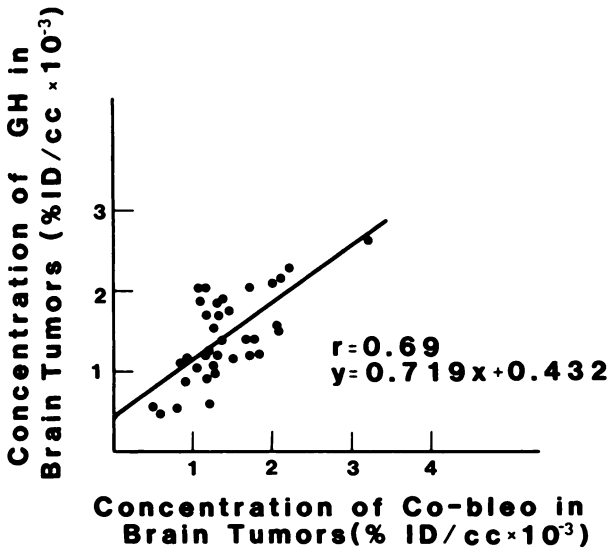

FIGURE 3
Correlation between the concentration of Co-bleo and GH in nine brain tumors at 30 min, 120, 240, and 480 min postinjection.

It is a principal rule of pharmacology that in order to exert beneficial effect a drug has to reach its site of action in a sufficient amount. This is critical in cancer chemotherapy where the dose-response curve is steep and a small reduction in the dose of the drug causes a significant reduction in tumor response (*20,21*). In animals with sensitive tumors, a reduction of only 20% in the optimal dose of a chemotherapeutic drug can cause a 50% reduction in response. In view of the large variation in uptake shown in our group of patients, even if a high dose of a chemotherapeutic drug is administered, only a limited amount, in some patients, will reach the tumor and thus the drug will be ineffective in inducing remission. Although opening of the blood tissue-barrier should probably be undertaken (*10*) in this group of patients, it would be unnecessary in patients showing high uptake. The results, then, indicate that treatment of brain tumors should be individualized according to the permeability of the particular tumor to be treated. It should be stressed, of course, that

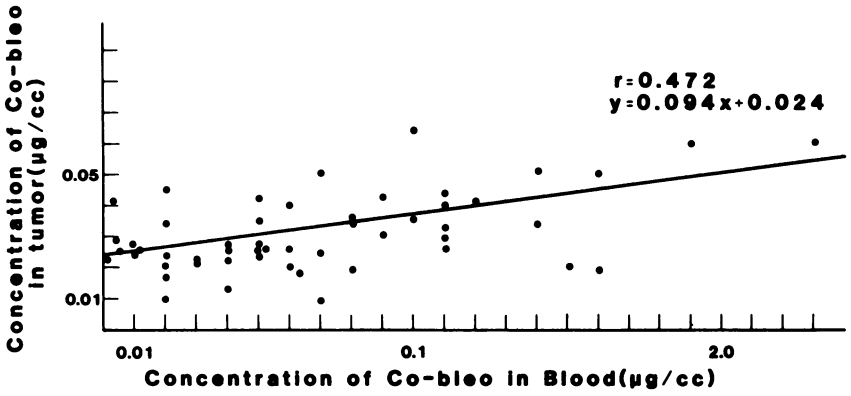

FIGURE 4
Correlation between the concentration of Co-bleo in 13 patients in the tumor and blood at 30, 120, 240, and 480 min postinjection.

significant uptake of any drug by a tumor does not in and of itself guarantee its effectiveness. In spite of high uptake the tumor cells may not be sensitive to the drug, but a drug has no chance at all of killing tumor cells if it does not enter the tumor in sufficient amounts. Although high uptake is not a sufficient condition for tumoricidal action, it is a necessary precondition.

Only a weak correlation was found between the uptake of GH and Co-bleo. This correlation was sought to determine whether simple commonly used agents such as GH could be utilized, instead of labeled chemotherapeutic drugs, to indicate the permeability of human brain tumors. Quantitative SPECT of the head with technetium agents could then be used before drug treatment as a simple procedure for evaluation of tumor permeability. Such simple scintigraphic agents have been used in nonquantitative studies as indicators of the permeability of brain tumors and for assessment of the effectiveness of methods used for the opening of the blood-brain barrier (*20*). Although GH uptake could not reliably represent Co-bleo uptake, further experience with quantitative SPECT is necessary to evaluate if other scintigraphic agents could achieve a better correlation with labeled drugs.

It is interesting that no correlation was found, when using either Co-bleo and GH, between the concentration of the labeled agents in the blood and in the tumor. It indicates that factors other than the blood level of the drug play a major role in determining the concentration of a drug in a tumor (*23,24*). These findings contrast with the classical textbook view: "(In most cases) . . . the concentration of a drug in the systemic circulation will be related to the concentration in the site of action. . ." (*23*). Apparently this is not the case in human brain tumors. We have shown (*16*) that the blood-tissue barrier depends on the length of the junction between the apposing membranes of adjacent endothelial cells. This and not the blood concentration is probably the most important factor determining the concentration of a drug in human brain tumors. This blood-tissue barrier has a different effectiveness in individual brain tumors, and it determines the bioavailability of the drug to the tumor. Bioavailability is the term used to indicate the extent to which a drug reaches its site of action (*18,23*). In our study it is expressed by the tumor cumulative concentration which indicates the amount of labeled bleomycin to which the tumor was exposed between 30 and 480 min. The TCC is the result of the interaction of all factors which determine the passage of a drug from the blood into the tumor:

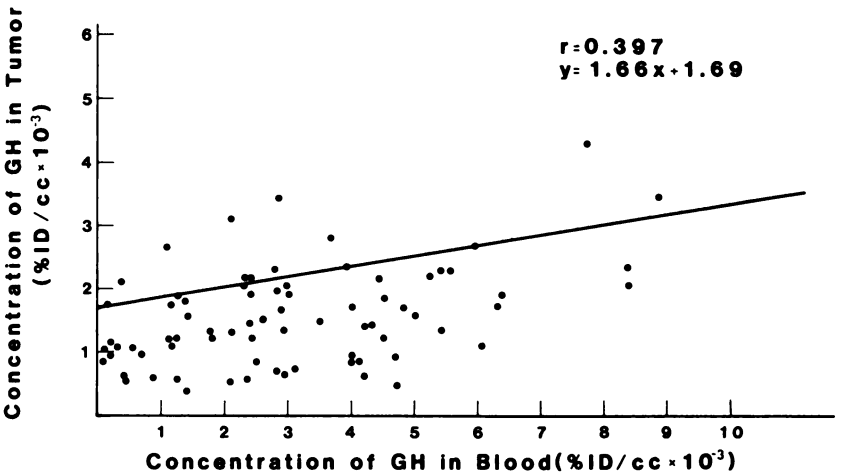

FIGURE 5
Correlation between the concentration of GH in 19 patients in the tumor and blood at 30, 120, 240, and 480 min postinjection.

the permeability coefficient of the capillaries with respect to the drug, the luminal surface area of the capillaries, the concentration of the drug in the plasma, and the length of time that the drug is found in the capillaries. In drugs which have a high extraction fraction, the blood flow of the tumor also plays a role.

Interest in neuro-nuclear medicine has recently been directed primarily to radiopharmaceuticals which cross the blood-brain barrier and are used to measure brain-blood flow, metabolism, and receptor distribution. Quantitative measurement of the effect of the blood-tissue barrier on drug uptake shows that nuclear medicine techniques can also have an impact on the treatment of human brain tumors which cannot be achieved by CT and NMR. The problem of improving the therapeutic effect of antineoplastic drugs on brain tumors was succinctly defined by Shapiro and Byrne (*9*): "The question remains as to the quantitative permeability of water-soluble chemotherapeutic agents into brain tumors." SPECT may be instrumental, in the future, in solving this question by measuring the uptake of drugs such as methotrexate and cisplatin which have been very effective in chemotherapy of tumors located in regions other than the brain.

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REFERENCES

1. Wilson CB, Levin VA, Hoshino T. In: Youmans JR ed. *Neurological surgery, Vol. 5: chemotherapy of brain tumors.* Philadelphia: W.B. Saunders Comp.; 1982:3065–3095.
2. Shapiro WR. Introduction. *Semin Oncol* 1986; 13:1–131.
3. Shapiro WR. Therapy of adult malignant brain tumors: what have the clinical trials taught us? *Semin Oncol* 1986; 13:38–45.
4. Levin VA, Landahl HD, Freeman-Dove MA. The application of brain capillary permeability coefficient measurements to pathological conditions and the selection of agents which cross the blood-brain barrier. *J Pharmacokinet Biopharm* 1976; 4:499–519.
5. Bar-Sella P, Front D, Hardoff R, et al. Ultrastructural basis for different pertechnetate uptake patterns by various human brain tumors. *J Neurol Neurosurg Psychiatry* 1979; 42:924–930.
6. Neuwelt EA, Specht DH, Hill SA. Permeability of human brain tumors to Tc-99m glucoheptonate and Tc-99m albumin. Implications for monoclonal antibodies. *J Neurosurg* 1986; 65:194–198.
7. Blasberg RG, Groothius DR. Chemotherapy of brain tumors: physiological and pharmacokinetic considerations. *Semin Oncol* 1986; 13:70–82.
8. Shapiro WR, Shapiro JR. Principles of brain tumor chemotherapy. *Semin Oncol* 1986; 13:56–69.
9. Shapiro WR, Byrne TN. In: Walker MD ed. *Oncology of the nervous system: Chemotherapy of brain tumors—basic concepts.* Boston: Martinius Nijhoff Publishers; 1983:65–98.
10. Neuwelt EA, Frenkel EP. Is there a therapeutic role for blood brain barrier disruption? *Ann Intern Med* 1980; 93:137–139.
11. Levin VA, Patlak L, Landahl HD. Heuristic modeling of drug delivery to malignant brain tumors. *J Pharmacokinet Biopharm* 1980; 10:257–296.
12. Blasberg RG, Groothuis DR, Molnar P. Application of quantitative autoradiographic measurements in experimental brain tumors. *Semin Oncol* 1981; 1:203–221.
13. Groothuis DR, Molnar P, Blasberg RG. Regional blood flow and blood to tissue transport in five brain tumor models. Implication for chemotherapy. In: Rosenblum M, Wilson C, eds. *Progress in experimental tumor research, Vol. 27: Brain tumor biology.* Basel: S. Karger, 1984:132–153.
14. Front D, Iosilevsky G, Frenkel A, et al. In vivo quantitation of radiopharmaceutical uptake by human meningiomas using single photon emission computed tomography (SPECT). *Radiology* 1987; 164:93–96.
15. Nieweg OE. Co-57 Bleomycin scintigraphy. Thesis. Groningen: Druckerij van Denderen; 1983:75–82.
16. Front D, Israel O, Kohn S, et al. The blood tissue barrier of human brain tumors. Correlation of scintigraphic and ultrastructural findings: concise communication. *J Nucl Med* 1984; 25:461–465.
17. Levin VA, Landahl HD, Freeman-Dove MA. The application of brain capillary coefficient measurements to pathological conditions and the selection of agents which cross the blood brain barrier. *J Pharmacokinet Biopharm*, 1976; 4:499–519.
18. Zimm S, Collins JM, Riccardi R, et al. Variable bioavailability of oral mercaptopurine. *N Engl J Med* 1983; 308:1005–1009.
19. Lyman S, Vijani B, Renner K, et al. Properties of the initial reaction of bleomycin and several of its metal complexes with Ehrlich cells. *Cancer Res* 1986; 46:4472–4478.
20. Frei E III, Canellos GP. Dose: a critical factor in cancer chemotherapy. *Am J Med* 1980; 69:585–594.
21. DeVita VT. Editorial: Dose-response is alive and well. *J Clin Oncol* 1986; 4:1157–1159.
22. Neuwelt EA, Specht HD, Howieson J, et al. Osmotic blood-brain barrier modification: clinical documentation by enhanced CT scanning and/or radionuclide brain scanning. *Am J Roentgen* 1983; 141:829–835.
23. Benet LZ, Sheiner T. Pharmacokinetics: The dynamics of drug absorption, distribution and elimination. In: Goodman-Gillman A, Goodman-Gillman LS, et al, eds. *The pharmacological basis of therapeutics*, 7th edition. New York: MacMillan; 1985:22.
24. Chabner B. Clinical pharmacokinetics and drug monitoring. In: Chabner B, ed. *Pharmacologic principles of cancer treatment.* Philadelphia: W.B. Saunders Comp,; 1982:100–108.