INTRATHECAL CHEMOTHERAPY: BRAIN TISSUE PROFILES AFTER VENTRICULO-CISTERNAL PERFUSION

R. G. BLASBERG, C. PATLAK AND
J. D. FENSTERMACHER

Membrane Transport Section, Division of Cancer Treatment, National Cancer Institute, and the Theoretical Statistics and Mathematics Section, National Institute of Mental Health, Bethesda, Maryland

ABSTRACT

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Ventriculocisternal perfusions with five isotopically labeled drugs were performed in the rhesus monkey and the resultant tissue diffusion concentration profiles in caudate nucleus were analyzed. The periventricular distribution space with respect to perfusate concentration was measured and expressed as microliters per 100 mg wet weight: hydroxyurea = 56; methotrexate = 27; thiotepa = 28; 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) = 64 and cytosine arabinoside >170. The apparent diffusion constants in caudate nucleus were determined for hydroxyurea and methotrexate (2.0 and 1.2×10^{-6} cm^2/sec, respectively); capillary permeability expressed as an extracellular space-transcapillary exchange half-time was estimated to be greater than 2 hours for both compounds. The extracellular fluid-transcapillary half-time was measured for thiotepa and BCNU (1.0 and 0.8 minute, respectively). Cytosine arabinoside continued to be concentrated by periventricular caudate nucleus during the course of perfusion; perfusate clearance measurements suggest a low capillary permeability. The apparent parenchymal diffusion constant and the capillary permeability of a drug in brain are discussed and are considered useful parameters for predicting drug levels after intrathecal administration.

Chemotherapeutic drugs have been utilized for some time in the treatment of central nervous system (CNS) malignancies (Shapiro and Ausman, 1969; Broder and Carter, 1972). The best therapeutic results have been achieved with metatastic tumors primarily involving the meninges such as the leukemias (Rieselbach et al., 1962a; Aur et al., 1971). The treatment of solid tumors involving brain parenchyma with chemotherapeutic compounds generally has been less successful (Rubin et al., 1966; Newton et al., 1968; Wilson and Hoshino, 1969). A major problem in treating CNS malignancy may be achieving an effective drug concentration at the tumor site for a given period of time. This topic has been the subject of two recent reviews (Broder and Rall, 1972; Fenstermacher and Blasberg, 1974).

The brain is a unique organ in that it is bathed by two accessible fluids; blood and cerebrospinal fluid (CSF). These fluids provide two routes which are clinically available for

drug administration to the brain. The brain levels reached after intravascular or systemic administration have been studied for a variety of compounds (Mayer *et al.*, 1959; Rall and Zubrod, 1962; Shanker, 1964). There is considerably less information available for drug distribution in the brain after administration into the CSF (Rieselbach *et al.*, 1962b; Korobkin *et al.*, 1968) and few studies which compare systemic and subarachnoid or intraventricular routes of drug administration (Fishman and Cristy, 1965; Lehrer *et al.*, 1973). For the chemotherapist who is trying to treat CNS disease, basic information on the movement of drugs from CSF to brain tissue are needed to aid in his choice of routes—blood or CSF—by which a drug may be most effectively delivered to a particular site in the CNS.

This paper will concern itself with the cerebrospinal fluid route of drug administration. Five different chemotherapeutic drugs, hydroxyurea, methotrexate, cytosine arabinoside, thiotepa and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) were studied. The brain tissue diffusion concentration profiles of each drug were determined after ventriculocisternal perfusion in the rhesus monkey. The following parameters were measured for each drug: 1) brain tissue level; 2) apparent rate of diffusion into the brain parenchyma from the ventricular surface; 3) permeability to brain capillaries; and 4) clearance from the ventriculocisternal perfusate. Some parameters which affect drug concentrations in the brain after intrathecal administration are discussed.

Methods

Technique. A unilateral ventriculocisternal perfusion in young adult rhesus monkeys, weighing 3 to 4 kg, was performed as described previously (Fenstermacher, 1972). The perfusions were from 15 minutes to 4 hours in duration. Two radioactively labeled compounds were used in all of the perfusions. One of the two labeled compounds was always an extracellular, capillary impermeable compound such as ^{3}H-sucrose or ^{14}C-ethylenediamine tetraacetate (EDTA)-Ca which served as a reference compound. The other labeled compound was one of the five drugs. An initial perfusion rate of 0.4 ml/min was used during the first 5 minutes to rapidly flush the ventricular CSF. The subsequent perfusion rate was 0.167 ml/min, 0.197 ml/min, and 0.246 ml/min for the 4-hour, the 1-hour, and the 30- and 15-minute perfusion periods, respectively. The perfusate was warmed to

approximately 37°C before entering the lateral ventricle. The cisternal outflow cannula was kept 5 cm below the cisterna magna to minimize bulk flow of perfusate over the hemispheres and into the systemic circulation *via* the normal pathways of CSF absorption. Throughout the perfusion period, samples of perfusate, cisternal effluent and plasma were obtained. At the end of the experimental period, the animal was killed by the intravenous injection of 2 ml of pentobarbital (360 mg/ml of Lethane), the scalp was reflected and the calvarium was removed. The brain was rapidly removed and bisected along the midline. Both hemispheres were immediately chilled in liquid nitrogen until firm, but not brittle (15–30 seconds). The time elapsed from death to brain cooling was 2 to 3 minutes. Coronal sections of the perfused hemisphere were made and rectangular blocks of caudate nucleus perpendicular to the ventricular surface were taken. The caudate nucleus was studied because its ventricular surface is well perfused by the inflow needle placement in the lateral ventricles, its thickness (0.5–0.8 cm) is such that a series of 7 to 10 serial sections of visibly homogenous tissue can be obtained, and its CSF border is smooth and reasonably flat. The caudate tissue blocks were then cut into a series of nine 0.5 mm thick slices parallel to the ependymal surface by using a special ten-blade knife. Throughout the slicing procedure, the coronal sections and caudate blocks were kept frozen by repeated dipping in liquid nitrogen. Each slice was placed in a tared counting vial and weighed. The samples of tissue, perfusate, cisternal outflow and plasma were digested in a tissue solubilizer (NCS, Amersham/Searle Corporation, Des Plaines, Ill.). Eighteen milliliters of a toluene based liquid scintillation fluid (Liquifluor, New England Nuclear Corporation, Boston, Mass.) were added to each sample vial. The radioactivity and isotope separation of each sample were determined by double labeled liquid scintillation spectroscopy with a Packard 4322 or Packard 3375 spectrometer using the appropriate background and quench corrections; the latter was determined by the external standard technique. The radioactively labeled compounds used were in tracer quantities; perfusate activity was 1 to 2 μc/ml for ^{14}C-compounds and 2 to 4 μc/ml for ^{3}H compounds: 1) hydroxyurea[^{14}C], New England Nuclear, 3.6 mc/mmol, 97% purity; 2) methotrexate[3,5-^{3}H] sodium salt, Dhom, 10 mc/mmol, repurified by chromatography (Oliverio, 1961), 99% purity; 3) thiotepa [tris(1-aziridinyl)phosphine sulfide[^{32}P], Amersham/Searle, 2.5 mc/mmol, 98% purity; 4) BCNU [1,3-bis(2-chloroethyl[U-^{14}C])-1-nitrosourea], Monsanto Research Corp., Dayton, Ohio, 10.5 mc/mmol, 98% purity; 5) cytosine arabinoside (cytosine-B-D-arabinofuranoside[G-^{3}H]), New England Nuclear, 11 mc/mmol, 99% purity; 6) EDTA-Ca (ethylenediamine tetraacetic acid[2-^{14}C]), New England Nuclear, 3.4

mc/mmol, 99% purity; a molar equivalent of $Ca(OH)_2$ was added to bind with EDTA and neutralize the acid; 7) sucrose, α-D-glucopyranosyl-β-D-fructofuranoside, fructose[1-^{3}H], New England Nuclear, 5.2 mc/mmol, purity 99.5%.

The purity of the radiochemicals was rechecked at the conclusion of random experiments by determining the diffusion profile and calculating the diffusion constants from the cisternal effluent collection in agar. The technique used for these diffusion studies was presented by Levin et al. (1970). Radioactivity was determined in a manner similar to that for tissue samples described above. The linearity of the agar diffusion profiles on inverse complimentary error function paper was checked and the diffusion constants (D_a) were determined (Schantz and Lauffer, 1962). Purity was estimated to be greater than 95% for each compound on the basis of the agar diffusion profile.

In this study, the chemical nature of the isotopically labeled compounds in the tissue itself was not determined. The tissue concentration for each tracer compound is presented as drug-derived radioactivity without specific reference to the chemical form or forms present in the tissue. BCNU, for example, is unstable in aqueous media and has been shown to have a very short chemical half-life in blood, plasma, CSF and saline (Montgomery et al., 1967). In the BCNU experiments reported here, the major portion of the radioactivity in the tissue probably is no longer associated with the parent compound but rather with one or several of its metabolites.

Data analysis. All of the isotopic data from the tissue and fluid samples were expressed in terms of tracer concentrations (counts per minute per milligram wet weight). The tissue concentration profiles (counts per minute per milligram or normalized counts per minute per milligram vs. distance from the perfusion surface) were plotted on both an inverse complimentary error function graph and a semilog graph. An inverse complimentary error function plot linearizes a one-dimensional diffusion, or kinetically similar, process (Schantz and Lauffer, 1962) and has been applied to brain tissue concentration profile analysis after ventriculocisternal perfusion (Katzman et al., 1968; Fenstermacher et al., 1970; Pollay and Kaplan, 1970). A semilog plot of the data was used to quantitate brain capillary permeability in terms of an extracellular fluid-transcapillary exchange half-time for those compounds which have relatively high rates of capillary exchange (Patlak and Fenstermacher, 1975). The distance from the ependymal surface to the midpoint of the first slice was calculated from the ratios of the first and second slices weights times one-half the distance between the knife blades. The distance between the midpoints of the second through eighth slice was determined by the distance between the knife blades. A tissue background was determined

from a distant tissue slice and/or from a plateau of the tissue concentration profile and subtracted from the raw data. The raw data were handled by a computer program which generated the tissue concentration profiles on both an inverse complimentary error function graph and a semilog graph with the aid of a Hewlett-Packard 7200A graphic plotter. For semilog analysis, the normalized tissue concentrations (C_x/C_o) were calculated from the measured tissue concentration (C_x) and from the subependymal concentration (C_o), the concentration at the tissue-fluid border. C_0 was determined for the semilog analysis by extrapolation of a least squares fit line through the experimental data points to its ordinate intercept ($x = 0$). The inverse complimentary error function analysis was more complex. The computer program systematically varied the value of C_0 and generated the respective series of quotients (C_x/C_o) for the data points to obtain the value for C_0 which yielded the best linear regression fit of the quotients on an inverse complimentary error function plot from which the tissue diffusion constant (D_b) was calculated (Schantz and Lauffer, 1962).

The tissue space of the drugs was calculated from both types of graphic analysis and is defined as the subependymal tissue concentration (C_o) divided by the inflow perfusate concentration and expressed as microliters per 100 mg wet weight.

The inverse complimentary error function plot of the extracellular marker, EDTA-Ca or sucrose, was analyzed first. If the series of points yielded a straight line and if the tissue space and tissue diffusion constant calculated by the computer program were reasonable based on previous results, analysis of the drug data for these tissue samples was performed. If these criteria were not met for the extracellular marker, the experiment was rejected on technical grounds; our rejection rate was approximately 20%. The justification for this approach is based on the observation that an extracellular compound moves from the cerebrospinal fluid into and through the brain by diffusion through the extracellular fluid (ECF) and does not significantly "leak" into the cerebral circulation or brain cells during the course of perfusion. Since the results with sucrose and EDTA-Ca as the extracellular marker compound (spaces and diffusion constants) were comparable and studies using sucrose have been published (Fenstermacher et al., 1970; Levin et al., 1970), only the EDTA-Ca data will be presented.

If a linear profile on the inverse complimentary error function graph was obtained for the drug data, the diffusion constant of the drug in caudate nucleus was calculated by the computer program from the slope of the plot as described above. If the tissue profile of the drug on the inverse complimentary error function graph was curvilinear upward, the semilog graph was analyzed. The semilog graph of these data
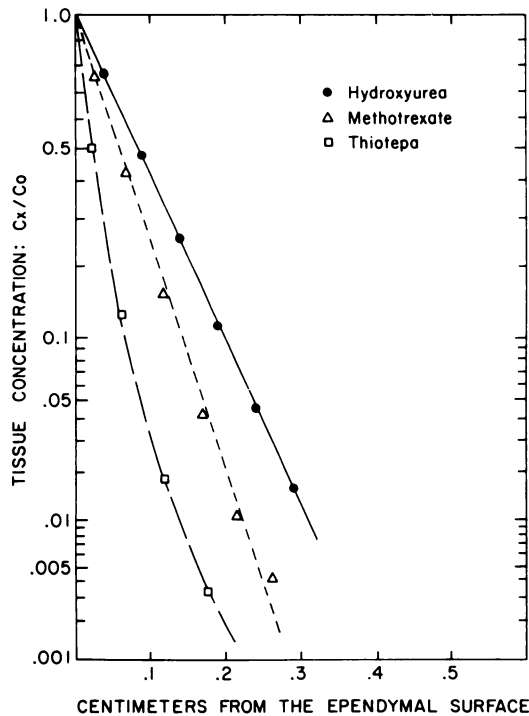
FIG. 1. Inverse complimentary error function graph showing the tissue concentration profiles of hydroxyurea, methotrexate and thiotepa found in separate experiments after 1 hour of ventriculocisternal perfusion.

generally yielded a straight line providing the length of perfusion was long enough for the tissue to reach a "steady state" profile. If a linear profile on a semilog graph was obtained, the capillary transfer rate of the drug, expressed as the ECF-transcapillary exchange half-time, was calculated from the slope of the plot using an approach developed by Patlak and Fenstermacher (1975). The equation for the steady state is:

$$C_x/C_0 = S \exp[-\sqrt{k_p/D_b} \cdot x] \qquad (1)$$

where S is a proportionality constant which contains terms involving the permeability of the ependymal layer, k_p is the capillary exchange or transfer coefficient and x is the distance from the perfused surface to the sampling site. In this paper, the capillary exchange coefficient (k_p) will be presented as the ECF-transcapillary exchange half-time ($T_{1/2}$); the relationship between k_p and $T_{1/2}$ is $T_{1/2} = \ln2/k_p$.

Results

Tissue profile. Typical brain tissue concentration profiles of five drugs measured in caudate nucleus after a 1-hour ventriculocisternal perfusion are presented in figures 1 and 2. Each

point represents a measured tissue concentration ratio (C_x/C_0) and distance (x) from the perfusion surface. Figure 1 is an inverse complimentary error function graph. Methotrexate and hydroxyurea show a linear relationship between tissue concentration ratio and distance from the ventricular (perfused) surface on an inverse complimentary error function graph, whereas the thiotepa plot is curvilinear, concave upward. Figure 2 is a semilog graph. Cytosine arabinoside, thiotepa and BCNU show a linear relationship between tissue concentration ratio and distance from the ventricular surface on a semilog graph; on the other hand, the hydroxyurea plot is curvilinear, concave downward.

Tissue space. Table 1 gives the brain tissue space measured in the caudate nucleus after two different perfusion times for each of the five drugs. The brain tissue space of EDTA-Ca is included as a measure of extracellular space. For four of the five compounds, the tissue spaces were essentially constant at their respective perfusion times. Cytosine arabinoside tissue space was significantly higher in the 4-hour experiments than in the 1-hour experiments. The cytosine arabinoside tissue space is larger
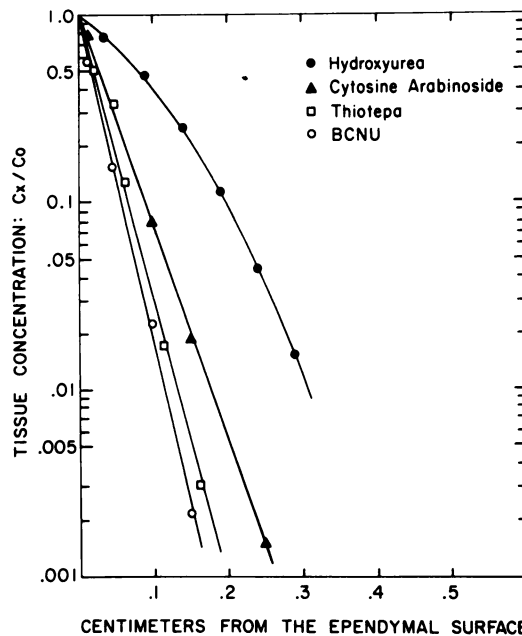

FIG. 2. Semilog graph showing the tissue concentration profiles of hydroxyurea, cytosine arabinoside, thiotepa and BCNU observed in separate experiments after 1 hour of ventriculocisternal perfusion.

TABLE 1

Brain tissue space after ventriculocisternal perfusion[a]

Drug	Mean Tissue Space at Varying Lengths of Time of Perfusion			
	$^1\!/_4$ hr	$^1\!/_2$ hr	1 hr	4 hr
	cpm/100 mg of tissue ÷ cpm/μl of perfusate ± S.E.			
Hydroxyurea			55.3 ± 4.1 (7)	57.5 ± 2.2 (7)
Methotrexate			25.0 ± 1.5 (5)	29.8 ± 2.5 (5)
Thiotepa	28.6 ± 2.5 (7)		28.3 ± 4.8 (6)	20.3 ± 5.5 (4)
BCNU		66.1 ± 0.8 (5)	63.0 ± 7.7 (7)	
Cytosine arabinoside[b]			171 ± 11 (6)	289 ± 25 (5)
EDTA-Ca			15.3 ± 0.4 (13)	15.2 ± 0.3 (9)

[a] Measured in caudate nucleus. The number of animals in each group is shown in parentheses.

[b] The difference in the tissue space between the 1- and 4-hour experiment is significant at $P < .01$ level.

TABLE 2

Diffusion constants in brain and agar[a]

Drug	Diffusion Constant		
	Caudate nucleus (D_b)		Agar (D_a)
	1-hr Expt.	4-hr Expt.	
	$cm^2/sec \times 10^{-6} ± S.E.$		
Hydroxyurea[b]	2.0 ± 0.2 (7)	1.5 ± 0.1 (7)	13.6 ± 0.3 (6)[c]
Methotrexate[b]	1.2 ± 0.1 (5)	0.59 ± 0.04 (5)	5.26 ± 0.07 (6)[c]
Thiotepa			9.84 ± 0.23 (3)
BCNU			14.3 ± 0.2 (5)
Cytosine arabinoside			8.45 ± 0.07 (3)
EDTA-Ca[b]	2.5 ± 0.1 (13)	2.1 ± 0.1 (9)	6.23 ± 0.09 (6)[c]

[a] Measured in caudate nucleus. The number of experiments is shown in parentheses.

[b] The difference in the diffusion constant between the 1- and 4-hour experiments is significant at $P < .05$ level.

[c] The difference in the diffusion constants in caudate nucleus and agar is significant at $P < .001$ level.

than either the extracellular space or the water space (80%). The tissue space of hydroxyurea, methotrexate, thiotepa and BCNU are greater than the tissue EDTA-Ca space.

Inverse complimentary error function analysis. The experimentally determined diffusion constants (D_b) of hydroxyurea, methotrexate and EDTA-Ca in brain after 1- and 4-hour perfusions are presented in table 2. The average of the diffusion constants was lower for the 4-hour experiment in comparison to the 1-hour experiment and this decrease was statistically significant. The diffusion constants (D_a) of all five drugs plus EDTA-Ca were also determined in 2% agar gel at 37°C (table 2). It is apparent from the ratio D_b/D_a, that diffusion is more rapid in agar than it is in the brain (table 3). The ratio D_b/D_a for EDTA-Ca is considerably higher than that of hydroxyurea or methotrexate.

Semi-log analysis. The preceding data were for two drugs and an extracellular marker compound which do not cross brain capillaries readily. For the drugs which exchange rapidly across brain capillaries and enter the systemic circulation, a different type of analysis is possible. Table 4 presents the brain ECF-transcapillary exchange half-times for thiotepa and BCNU measured in caudate nucleus after two different periods of perfusion. The capillary half-times were essentially identical for both periods of perfusion. Since both thiotepa and BCNU readily permeate cellular and capillary membranes, the appropriate brain diffusion constant (D_b) to be used in equation 1 is assumed to be equal to the diffusion constant of each drug in water (D_a). With this approximation, ECF-transcapillary exchange half-times of 0.8 and 1.0 minute were obtained for thiotepa and BCNU, respectively.

A precise value for the extracellular space (ECS) half-time of cytosine arabinoside is not possible from this type of analysis since the extracellular and intracellular compartments were not in equilibrium; the tissue space was increasing between 1 and 4 hours (table 1).

Length of perfusion. Increasing the length of ventriculocisternal perfusion increased the depth of hydroxyurea and methotrexate penetration. For example, with hydroxyurea a tissue concentration of $C_x/C_0 \geq 0.01$ extends 0.3 cm into the tissue from the ependymal surface after a 1-hour perfusion, whereas the same concentration extends 0.6 cm into the tissue after a 4-hour perfusion (fig. 3).

In the case of thiotepa and BCNU, increasing the length of perfusion did not increase the depth of drug penetration. The tissue concentration profiles of thiotepa after a 15-minute and 4-hour perfusion are essentially identical (fig. 4). A steady state between labeled material diffusing into the brain and leaving the brain through the capillaries has been established in less than 15 minutes.

Perfusate clearance. The net flux for each of the drugs from the ventriculocisternal perfusate

to the whole animal during a 1-hour perfusion is presented in table 5. The value for EDTA-Ca is also presented as a reference molecule since it has been shown to have negligible capillary permeability and remain predominantly in the extracellular space. The net fluxes of methotrexate, hydroxyurea and cytosine arabinoside from the ventriculocisternal perfusate are fairly similar and 4 to 5 times greater than that of the reference compound EDTA-Ca. In contrast, the net fluxes of thiotepa and BCNU from the perfusate are much greater than the above compounds. The blood levels for the latter two drugs, also, are very high relative to the other compounds (table 5).

Discussion

Cancer chemotherapeutic drugs have been administered through the CSF in the treatment of both primary and secondary brain tumors. However, the concentration profile of these drugs in the brain after CSF administration has not been determined previously nor have the physiological and pharmacological parameters which effect drug concentration in the brain been examined. Some of these parameters—diffusion through the ECF, loss across the capillaries into the blood, and uptake by cells and/or binding—will be presented using the results found with the five drugs in this study.

Diffusion through brain extracellular fluid. If a drug moves from the CSF into brain tissue and subsequently diffuses through the ECF without significant cellular uptake or capillary loss, a graph of C_x/C_0 vs. x for that drug should give a straight line on inverse complimentary error function paper and the apparent diffusion constant of the drug within the tissue (D_b) can be determined. Diffusion profile analysis also is appropriate if the drug moves through the ECF by diffusion and equilibrates relatively rapidly between the intracellular and extracel-

TABLE 3

Comparison of the diffusion constants in brain and agar

Drug	D_b/D_a Ratio[a]	
	Observed	Calculated[b]
	$cm^2/sec \times 10^{-6} \pm S.E.$	
Hydroxyurea	0.14	0.11
Methotrexate	0.23	0.24
EDTA-Ca	0.40	

[a] Ratio of the diffusion constant in brain, D_b (1-hour experiment, table 2) to the diffusion constant in agar, D_a (table 2).

[b] Calculated ratio D_b/D_a; see "Discussion," equation 3.

TABLE 4

Brain ECS-transcapillary exchange half-time[a]

	Mean ECS-transcapillary Half-time at Varying Lengths of Time of Perfusion			
	$\frac{1}{4}$ hr	$\frac{1}{2}$ hr	1 hr	4 hr
	min $\pm$ S.E.			
Thiotepa	0.87 $\pm$ 0.05 (7)		1.03 $\pm$ 0.04 (6)	1.13 $\pm$ 0.05 (4)
BCNU		0.81 $\pm$ 0.06 (5)	0.78 $\pm$ 0.05 (7)	

[a] Measured in caudate nucleus. The number of animal experiments is listed in parentheses.

lular compartments but has negligible capillary permeability and negligible brain tissue metabolism. Two drugs used in this study, methotrexate and hydroxyurea, appear to be treated by the system in the latter fashion, and thus their apparent diffusion constants in brain

could be determined from the slope of the tissue profile on an inverse complimentary error function graph.

The experimentally determined diffusion constants of hydroxyurea and methotrexate decreased slightly from the 1- to the 4-hour experiment (table 2). A smaller value for D_b will be obtained with longer periods of perfusion if there is a small loss of drug from the ECF across the capillaries (Patlak and Fenstermacher,
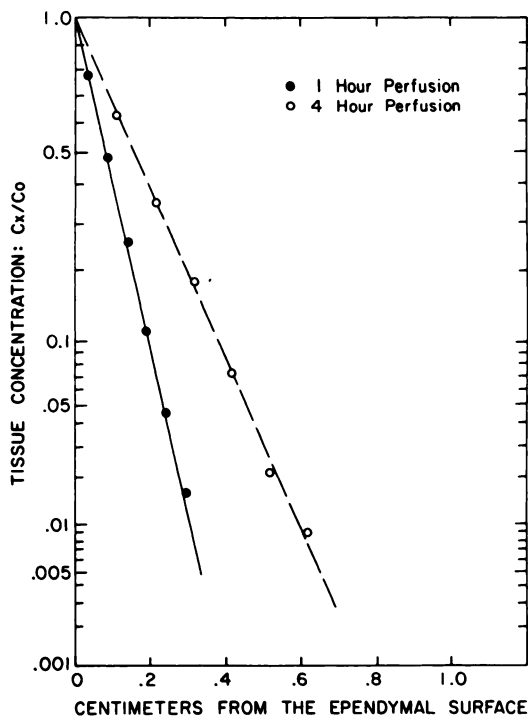

Fig. 3. Inverse complimentary error function graph presenting the tissue concentration profile of hydroxyurea in experiments of 1-hour and 4-hour duration.
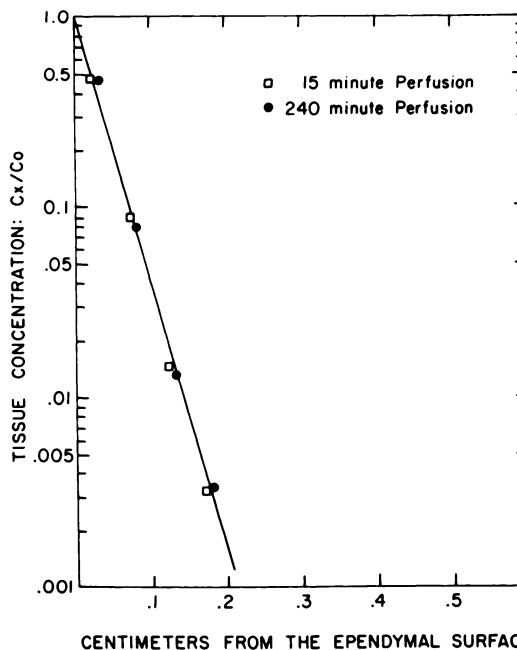

Fig. 4. Semilog graph showing the tissue concentration profile of thiotepa measured in experiments of 15 and 240 minutes duration.

TABLE 5

Drug clearance from the ventriculocisternal perfusate during a 1-hour perfusion

Drug	Outflow/Inflow Ratio[a]	Perfusate Clearance[b]	Plasma/Perfusate ratio[c]
		$\mu l/min$	$\times 10^{-4}$
EDTA-Ca	0.83 ± 0.01 (13)	4	1.3
Hydroxyurea	0.80 ± 0.01 (8)	14	1.4
Methotrexate	0.79 ± 0.02 (4)	16	.64
Cytosine arabinoside	0.77 ± 0.01 (10)	22	3.2
Thiotepa	0.57 ± 0.01 (10)	113	21.7
BCNU	0.52 ± 0.02 (7)	145	15.2

[a] Average of outflow to inflow perfusate ratios ± S.E.: the number of experiments is shown in parentheses.

[b] Perfusate clearance was calculated by the equation of Heisey *et al.* (1962) using a constant inflow rate of 197 $\mu l/min$ and an average CSF production rate of 36 $\mu l/min$.

[c] Average ratio of plasma/perfusate radioactivity after a 1-hour experiment.

1975). From the limits of the experimental method and analysis employed in this study, we can say that ECF-transcapillary half-time for hydroxyurea and methotrexate must be in excess of 2 hours; the data do not permit a more precise estimate. The diffusion constant determined from the shorter (1-hour) experiment is less affected by the capillary loss and is closer to the true extracellular space value. The 1-hour values of the distribution space and diffusion constant (D_b) are presented in tables 1 and 2, respectively.

A comparison of the apparent diffusion constant in brain (D_b) with the diffusion constant in 2% agar–98% saline gel (D_a), is given in table 3 and in several publications from this laboratory (Fenstermacher et al., 1970; Levin et al., 1970). Compounds which do not cross capillary or cell membranes to a significant extent have similar D_b/D_a ratios. This ratio, which we will denote as ECF diffusion coefficient ratio (Q), has been found experimentally to have a value of about 0.35 to 0.45 for extracellular marker compounds such as sucrose, inulin and EDTA-Ca in dog and monkey caudate nucleus (Fenstermacher et al., 1970; Patlak and Fenstermacher, 1975; this study). The Q value for EDTA-Ca determined in this study was 0.40 (table 2). One possible explanation for this reduction in the apparent rate of diffusion through brain tissue and the similarity in the magnitude of this reduction for extracellular marker compounds of different molecular weights and agar diffusion constants is the tortuosity of the extracellular space. Thus, it may be that molecules which move exclusively through the extracellular fluid follow a long tortuous path—the extracellular space—in their course through brain tissue. A different mechanism, trapping by dead-end pores, has been suggested by Suenson et al. (1974) to explain their results with cardiac muscle. Such a system could also be involved, in part or in total, for the caudate nucleus; however, our present data do not provide a way of choosing between these two models. Under conditions where there are no dead-end pores, the relationship between the tissue D_b and the water or agar D_a for an extracellular marker compound is:

$$D_b/D_a = Q = 1/\lambda^2 \qquad (2)$$

where λ is the tortuosity factor. On the basis of this assumption and the results presented in

this paper and those cited above, λ approximates 1.6. Hence the true distance (microscopic) across which these materials are diffusing in the ECF is longer than the apparent distance (macroscopic) measured by the tissue sampling technique by approximately 60%.

Compounds which enter the ECF and do cross cellular and/or capillary membranes have lower D_b/D_a ratios than extracellular compounds (Fenstermacher et al., 1970). The ratio D_b/D_a for a drug which equilibrates fairly rapidly with cells but exchanges relatively slowly across brain capillaries and, in addition, moves into the tissue principally by diffusion through extracellular channels is a function of the tissue distribution volume (TS) of the compound and the size of the ECS as well as the tortuosity and/or the dead-end pores of the ECS which is expressed by the ECF diffusion coefficient ratio, Q (Fenstermacher et al., 1974):

$$D_b/D_a = ECS/TS \cdot Q \qquad (3)$$

In table 3 the ratio D_b/D_a "observed" is remarkably similar to the ratio D_b/D_a "calculated" with equation 3 for both methotrexate and hydroxyurea. This indicates that the experimentally determined value compares well with the value calculated from the model of extracellular diffusion with rapid brain cell-ECF exchange, i.e., rapid cell-ECF exchange in comparison to the rate of change of the ECF concentration during non-steady-state conditions. The correlation also suggests that linear or one-dimensional diffusion across cell membranes and through the intracellular fluid does not significantly contribute to the net movement of these drugs into the brain under non-steady-state conditions. The loss of methotrexate or hydroxyurea from the ECF to brain cells, however, diverts a significant amount of the drug from further diffusion through the ECS into the brain. The effect of an "intracellular sink" will be to reduce further the apparent D_b and the D_b/D_a ratio will be lower than that of an extracellular marker compound such as EDTA-Ca.

Several important points about the diffusional delivery of compounds to the CNS by way of the CSF can be seen from figures 1 and 3. Of the two drugs which do not appreciably leak out of ECF into the blood, hydroxyurea, the compound which has the higher D, penetrates more deeply than the larger compound, metho-

trexate (fig. 1). Figure 3 illustrates that the extent of diffusional entry into the tissue from the CSF of hydroxyurea is directly proportional to the square root of the perfusion time; a particular concentration of hydroxyurea will be found at a point twice as far into the tissue for a 4-hour perfusion than for a 1-hour experiment. Both figures 1 and 3 show that diffusional delivery over short distances (<0.1 cm) is rapid and efficient for both methotrexate and hydroxyurea, whereas diffusion over long distances (>0.4 cm) is slow and relatively ineffective for these same two drugs even after 4 hours of perfusion.

The preceding discussion has been concerned only with events up to the termination of the perfusion period. After the perfusion, three things will happen: 1) the concentration of drug in the cerebrospinal fluid will fall as it is being cleared from this fluid compartment; 2) the drug will continue to diffuse further into the brain and 3) the drug will also start to diffuse back into the cerebrospinal fluid as the cerebrospinal fluid concentration falls. These effects have been demonstrated experimentally with inulin (Rall, 1968). Further theoretical and experimental confirmation of these postperfusion events is in progress.

Effects of capillary permeability. Drugs which move across brain capillaries readily are in general either nonpolar (lipid soluble) or carrier mediated, whereas drugs which do not cross brain capillaries are usually polar (lipid insoluble) and not carrier mediated (Mayer *et al.*, 1959; Rall *et al.*, 1959; Rall and Zubrod, 1962; Oldendorf, 1971). Thiotepa and BCNU are representative of the first class of compounds. Methotrexate, hydroxyurea and cytosine arabinoside are representative of the second class of compounds.

When drugs that have high capillary permeability are perfused, the periventricular tissue rapidly reaches a steady state. In this situation, the net diffusion of drug into the brain from the CSF is balanced by an equal net loss of drug from the brain through the capillaries to the systemic circulation. Increasing the length of perfusion, therefore, will not change the tissue profile once a steady state has been reached; this was demonstrated graphically for thiotepa (fig. 4). Drugs which exchange readily across brain capillaries penetrate the brain to a very limited extent when perfused through the CSF

system. Drug levels in all but the first several millimeters of subependymal and subpial tissue will be low and will be primarily determined by the arterial blood level. Little or no advantage in drug delivery to the brain parenchyma can be obtained by the administration of a drug into the CSF if the drug rapidly equilibrates across cell and capillary membranes.

For the calculation of the ECF-capillary exchange half-times for thiotepa and BCNU, it was assumed that these drugs diffuse into the tissue equally through the intracellular and extracellular fluids, *i.e.*, brain cell membranes did not act as obstacles in the diffusional movement of BCNU and thiotepa within the brain parenchyma, and that their effective tissue diffusion constants were equal to their respective agar or water diffusion constants ($D_b = D_a$). If this assumption is incorrect and diffusion of these drugs into the brain occurs primarily through the ECF pathways, their diffusion constants in caudate nucleus may be obtained from the relationships $D_b = D_a \cdot Q$ and the ECF-transcapillary half-times given in table 4 should be multiplied by $1/Q$ (2.6).

A critical limitation on the accurate measurement of rapid ECF-transcapillary exchange (short capillary half-time) is imposed by capillary blood flow. Patlak and Fenstermacher (1975) calculated that measurements of capillary half-time by ventriculocisternal perfusion are significantly affected by capillary blood flow when the capillary exchange half-times of the caudate nucleus are around 5 minutes or less. A calculated half-time of 1 minute as found for BCNU and thiotepa indicates that the movement of these drugs between plasma and ECF is affected not only by the permeability of the capillaries but also by the rate of capillary blood flow.

The fractional loss or extraction (*E*) can be calculated from the equations developed by Patlak and Fenstermacher (1975) assuming a blood flow (*F*) of 1.0 mg/g-min and a volume distribution (*V_e*) of 0.8 ml/g of tissue:

$$E = k_p V_e / F \qquad (4)$$

where k_p is the capillary exchange coefficient as defined under "Methods." The calculated fractional extraction of thiotepa and BCNU is 0.55 and 0.69, respectively. For such high extraction fractions, the clearance of these compounds from brain tissue is strongly dependent on blood

flow. Assuming that the transfer constants of these compounds across the capillaries from ECF to blood and from blood to ECF are equal it is apparent that 50 to 70% of these two drugs would enter the brain from the arterial blood side during first passage through the brain capillaries.

Cellular uptake and metabolism. The effect of cell uptake and/or binding on the apparent rate of diffusion of a drug through the brain ECS (D_b) compared to that through water or agar (D_a) has been discussed. In the case where a drug is continually being taken up or concentrated by brain tissue, the subependymal and subpial neurons and/or glia are removing drug from the ECF and acting as a "sink" limiting the amount of drug which is "free" to diffuse further along through the ECF pathways to deeper brain cells. Cytosine arabinoside flux from CSF into the brain falls into this category. Although the tissue concentration profile of cytosine arabinoside is linear on a semilog graph, it cannot be analyzed as extensively as the other four drugs. The intracellular and extracellular compartments have not come into equilibrium during the experimental period (table 1). Nevertheless, the accumulation of cytosine arabinoside and/or a metabolic product by the subependymal tissue is evident.

Similarly, drug accumulation and/or metabolism by normal brain cells will affect the tissue profile of the administered or original form of the compound. In this respect, cellular uptake and metabolism may be as significant as capillary permeability in limiting the penetration of a compound from the CSF to deep brain sites.

Cerebrospinal fluid clearance. The clearance of drugs from the perfusate during a ventriculocisternal perfusion or from the CSF compartment after bolus administration must be considered before chemotherapeutic drugs are given by this route. Clearance does occur by bulk flow of entrained drug through the normal pathways of CSF absorption. Clearance rates by bulk flow can be calculated from the rate of CSF production and the volume of the CSF compartment. However, drug may also be cleared from the CSF by molecular exchange with and diffusion into the brain parenchyma and choroid plexuses. If the drug is also rapidly cleared from these tissues by movement across the capillaries and loss to the systemic circulation, a steep diffusion gradient will be main-

tained in the subependymal or subpial brain tissue. This is the case for BCNU and thiotepa; these drugs readily penetrate brain capillaries, maintain a steep diffusion gradient in the subependymal tissue and are cleared rapidly from the perfusate (table 2, fig. 2 and table 5, respectively). Those drugs which were cleared slowly, such as hydroxyurea and methotrexate, apparently cross the brain capillary complex quite slowly, also. Their somewhat greater clearance compared to that of the reference compound. EDTA-Ca, is due in part to their uptake and/or binding by brain and/or choroid plexus cells. Cellular uptake and/or binding also may be a significant factor in influencing the clearance of cytosine arabinoside from the ventriculocisternal perfusate.

The anticipated relationship between capillary permeability and perfusate clearance was demonstrated for BCNU and thiotepa. For these drugs or other compounds which have high brain capillary transfer coefficients (low ECF-transcapillary exchange half-times) the CSF would not serve as an effective route for drug delivery to distant subarachnoid sites and only very small amounts of drug would be expected to reach the brain by this route of administration. In this situation, most of the drug that reaches the brain after lumbar sac administration will be delivered by the systemic circulation. Drug will pass rapidly from the lumbar subarachnoid space into the systemic circulation by diffusion across spinal capillaries and re-enter more distant brain tissue by an equally rapid diffusion across the brain capillaries from the cerebral circulation.

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