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Putrescine Diffusion in Cat Brain and Capillary Permeability in Rat Brain: Relation to CSF Putrescine Levels in Brain Tumor Patients*

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Abstract—*Cerebrospinal fluid (CSF) putrescine (Pu) levels are extremely useful measures of active tumor growth in patients harboring medulloblastoma but not in patients harboring most supratentorial malignant gliomas. This study was designed to determine the diffusion coefficient* (D) *for Pu in cat brain and the capillary permeability coefficient* (P_c) *in rat brain to explain the failure of supratentorial gliomas to manifest a consistent increase in CSF Pu with progressive tumor growth. The* P_c *for Pu was found to be greater than the* P_c *for urea, while the apparent brain* D *for Pu was lower than that for urea. This implies that Pu crosses capillaries and enters cells more rapidly than urea, which would reduce the amount of Pu that might ultimately reach the CSF by diffusion from tumor. These data explain why CSF levels of Pu are correlated best in medulloblastomas—generally located adjacent to the CSF pathways—and why levels are correlated least in malignant supratentorial gliomas—usually located within the brain hemispheres, from which diffusion to the ventricles and into the CSF would be difficult.*

INTRODUCTION

THE POLYAMINES putrescine (Pu), spermidine (Sd) and spermine (Sp) are low molecular weight intracellular aliphatic amines involved in cell proliferation, concentrations of which are elevated in proliferating tissue [1–3]. Elevated levels of polyamines have been found in the physiological fluids of patients harboring a wide variety of solid tumors [1–4], which may be due either to the rapid proliferation of tumor cells or to the release of polyamines from dead and/or dying tumor cells.

Cerebrospinal fluid (CSF) polyamine levels, although not useful for screening to diagnose brain tumors [5], are of value in monitoring tumor progression or tumor regression that results from treatment of patients harboring medulloblastoma [3, 6], and may be the earliest indicator of tumor progression [4, 6]. But a recent study [7] showed that the elevation of CSF Pu in patients harboring supratentorial gliomas did not correlate with clinical status, histology, tumor volume, or extent of tumor necrosis, but did correlate with the proximity of the tumors to the CSF pathways. Elevation of CSF Pu levels in patients harboring medulloblastoma, and the absence of consistently elevated CSF Pu levels in patients harboring supratentorial malignant gliomas, may be due to the proximity of the former tumors to, and the distance of the majority of the latter tumors from, the CSF pathways.

Because diffusion of Pu from the tumor

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Abbreviations: Pu, Putrescine; Sp, spermine; CSF, cerebrospinal fluid; *D*, diffusion coefficient; P_c, capillary permeability; Sd, spermidine; SSA, 5-sulfosalicilic acid; ECF, extracellular fluid.

through brain parenchyma to the CSF and the transcapillary exchange of Pu are possible mechanisms to explain the fact that elevated Pu levels are not seen in the CSF of many patients harboring parenchymal malignant gliomas, we measured apparent brain tissue diffusion coefficient and brain capillary permeability for [2, 3-^{3}H] Pu in cats and rats, respectively..

MATERIALS AND METHODS

Cat brain diffusion profiles

[2, 2-^{3}H] Pu and [^{14}C] urea were purchased from New England Nuclear (Boston, Mass.).

Using a stereotactic procedure [8–10], mock CSF [11] containing the labeled compounds and Evans blue dye (used to verify the area of perfusion) was infused through one lumbar puncture needle inserted into the left ventricle (ventriculocisternal perfusion), or through two lumbar puncture needles inserted into the supracallosal space (subarachnoid cisternal perfusion). A needle was placed in the cisterna magna for CSF outflow.

Cats were sacrificed after 1 hr of perfusion; the brains were rapidly removed and chilled to medium hardness in liquid nitrogen. From coronal sections uniformly well-stained with Evans blue dye, rectangular strips of either cortex or caudate gray matter were sliced perpendicular to the surface. Each strip was sectioned into 0.75 mm-wide parallel slices with a multiblade knife and transferred to tared sterile bottles before being weighed. An 8% solution of 5-sulfosalicilic acid (SSA) was added to brain samples (5 μl SSA/1 mg sample), which were sonicated, stored on ice for 1 hr, and centrifuged. The CSF samples were treated in the same way without sonication.

The supernatant was fractionated using a Durrum D-500 amino acid analyser (Dionex Inst. Co., Sunnyvale, Calif.) as previously described [12]. The eluant was collected in 42 fractions (1.5 min each) on an Eldex fraction collector (Eldex Laboratories, Menlo Park, Calif.); 6 ml of PCS (Amersham-Searle, Arlington Heights, Ill.) were added to each fraction, and the samples were counted in a Hewlett–Packard Tri-Carb liquid scintillation spectrometer (Hewlett–Packard, Palo Alto, Calif.). Fractions corresponding to [^{14}C] urea and fractions corresponding to [2, 3-^{3}H] Pu were corrected for recovery, quench (external standard method) and dilution, and expressed as dpm/g for tissue samples and dpm/ml for fluids. In addition, the total radioactivity of each homogenized tissue was measured after solubilization with NCS (Amersham-Searle).

Brain diffusion profiles for [2, 3-^{3}H] Pu and [^{14}C] urea were obtained using a computer program supplied by Dr. C. S. Patlak (Theoretical Statistics and Mathematics Branch, NIMH, NIH, Bethesda, Md.) [13].

Rat brain capillary permeability study

These studies were done in ether-anesthetized adult male Fischer 344 rats weighing between 160 and 200 g using the technique of Levin *et al.* [8, 14]. [2, 3-^{3}H] Pu was injected in the femoral vein and eight blood samples were taken over 2 min from a cannula placed in the femoral artery. After sacrifice, one cortical and one subcortical tissue sample per hemisphere were obtained. The tissues were treated as described above.

After blood samples were centrifuged, each serum sample was added to 1.0 ml of 12% SSA, stored on ice for 1 hr, centrifuged, and treated as described above for the cat CSF samples.

The capillary permeability coefficient (P_c, cm/sec) was calculated using the formula

$$P_c = K_i 0.28\,(ICD)\,(BV)^{-1/2}. \qquad (1)$$

The intercapillary distance (ICD) in cm and the blood volume (BV) in ml/g were determined previously [8]. The rate of transcapillary exchange (K_i) was computed by the relationship

$$K_i = 0.93\,C(1 - PW)\,(AUC)^{-1}, \qquad (2)$$

where C = dpm/g tissue, PW = plasma water, and AUC = area under plasma curve during the experimental period, dpm . min/g plasma [14].

RESULTS

Figure 1 shows a typical profile for [2, 3-^{3}H] Pu and [^{14}C] urea diffusion in cats from ventricular CSF through the caudate nucleus. Table 1 summarizes the mean diffusion coefficients (D) measured in gray matter (cortex and/or caudate nucleus) for the two molecules. There was no difference between computed diffusion coefficients for caudate and cortex tissue, and the data were pooled. Because the value for [^{14}C] urea—the 'standard molecule'—at 1 hr (4×10^{-6} cm^2/sec) agrees with the value determined by Dr. J. D. Fenstermacher (Section on Membrane Transport, DTP, DCT, NCI, NIH, Bethesda,

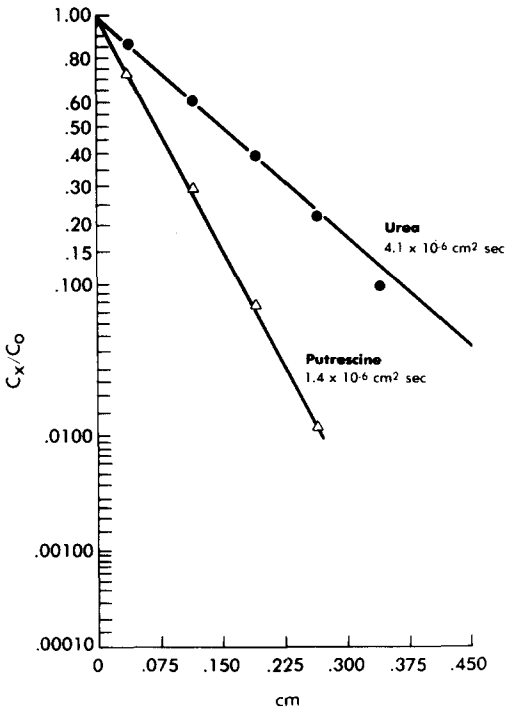

Fig. 1. Typical concentration profiles obtained with [2, 3-^{3}H] *Pu and* [^{14}C] *urea in cat brain tissue. Each point represents the ratio of concentration of the tracer in each tissue* (C_x) *to the theoretical or extrapolated concentration of tracer at the interface between tissue and CSF* (C_0) *plotted as a function of distance* (x) *from the CSF-tissue surface. The ordinate is the complementary ERFC (error function). D was computed by the method of Patlak* [8, 13].

MD), our determination of the [2, 3-^{3}H] Pu D is corroborated. No significant radioactivity was found in the fractions corresponding to Sd and Sp, suggesting that in a 1 hr period exogenous Pu is not significantly metabolized to Sd or Sp in the brain. No significant radioactivity was found in the pellet from tissue homogenates, which showed that [2, 3-^{3}H] Pu was not trapped in cell cytosol.

Table 1. Cat brain diffusion coefficients of putrescine and urea (cm^2/sec)

	[2, 3-^{3}H] Pu	[^{14}C] Urea
Mean	2.3×10^{-6}	4.0×10^{-6}
% S.E.	6	4
Number of profiles	12	12
Number of cats	5	5

Table 2 summarizes the mean brain capillary P_c for [2, 3-^{3}H] Pu in rat cortex and subcortex. The value of 1.5×10^{-5} cm/sec suggests that [2, 3-^{3}H] Pu readily crosses intact rat brain capillaries at a rate slower than tritiated water (1.6×10^{-4} cm/sec) [8], but faster than [^{14}C] urea (7.1×10^{-6} cm/sec) [14].

DISCUSSION

The relatively high capillary permeability of Pu in rat brain may partially explain the low diffusion coefficient of [2, 3-^{3}H] Pu measured in cat gray matter. Because Pu readily crosses capillaries, the amount of Pu that will diffuse across the brain through the extracellular fluid (ECF) is reduced (Fig. 2). In addition, the fact that Pu can enter cells further reduces the amount of Pu that can diffuse in the ECF of brain. The ratio of the apparent diffusion coefficient in cat gray matter, D_{App}, to the free water diffusion coefficient, D_W, reflects entry into the CSF and across capillaries; values of D_{App}/D_W below 0.40 but above 0.20 indicate intracellular and a small amount of transcapillary loss, whereas values below 0.20 indicate more extensive intracellular entry and transcapillary loss [10].

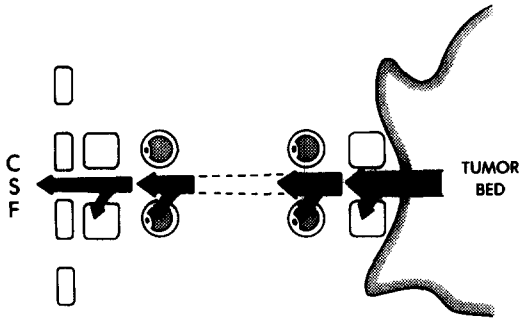

Fig. 2. This schematic drawing shows a large amount of Pu released by the tumor bed diffusing from the tumor into the brain and entering cells and crossing capillaries as it moves toward the CSF pathways. This pictorial representation may explain why the amount of Pu that ultimately reaches the CSF from a parenchymal tumor may not be sufficient to elevate CSF Pu levels in many patients.

Table 2. Rat brain permeability coefficients (P_c, cm/sec) *for* [2,3-^{3}H] *putrescine*

	Cortex	Subcortex	Mean brain
Mean	1.7×10^{-5}	1.4×10^{-5}	1.5×10^{-5}
% S.E.	13	8	9
Number of rats	5	5	5

Comparing the ratio for [^{14}C] urea yields a D_{App}/D_W of 0.27* and for [2, 3-^{3}H] Pu a value of 0.07.† Therefore, the lower ratio of D_{App}/D_W for [2, 3-$\frac{1}{m}$^{3}H] Pu indicates more rapid intracellular uptake and transcapillary loss compared to [^{14}C] urea.

Figure 3 is a hypothetical plot of Pu diffusion from a sphere (the tumor) within a larger sphere (the brain). To compute the profile, D was set at 1.3×10^{-5} cm^2/sec (0.4 times D_W), to account for the tortuosity of the ECF; the loss of Pu from the brain to blood was taken from equation (2) to be K_i(0.0924 min^{-1} for a $t_{1/2} = 7.5$ min). Figure 3 graphically demonstrates that, at equilibrium, very little Pu would be expected to diffuse far from the tumor. Therefore, tumor Pu levels would not produce an elevation in CSF levels unless the tumor were almost impinging on the ventricular system or the subarachnoid space.

Although cellular uptake of Pu as it diffuses from the tumor to the CSF further reduces the amount of Pu available to diffuse to the CSF, this does not appear to be as important as Pu loss across brain capillaries. This is supported by our observation that the permeability coefficient for [2, 3-^{3}H] Pu into S49 cells was 4.5×10^{-6} cm/sec, only 30% of the value reported here for permeability into brain capillaries [15].

Because supratentorial malignant gliomas are usually located within the hemispherical white matter, the Pu produced by the tumor cells must diffuse millimeters to centimeters before reaching the CSF. During diffusion, Pu crosses normal brain capillaries and enters cells. The quantity of Pu that reaches the CSF may not be sufficient to produce elevated CSF levels and therefore may not be used as a reliable indicator of tumor behavior. In patients harboring medulloblastoma, Pu levels are well correlated to tumor growth and cell burden because, in most patients, these tumors are located adjacent to the CSF pathways, and are highly proliferative and would be expected to produce high levels of Pu.

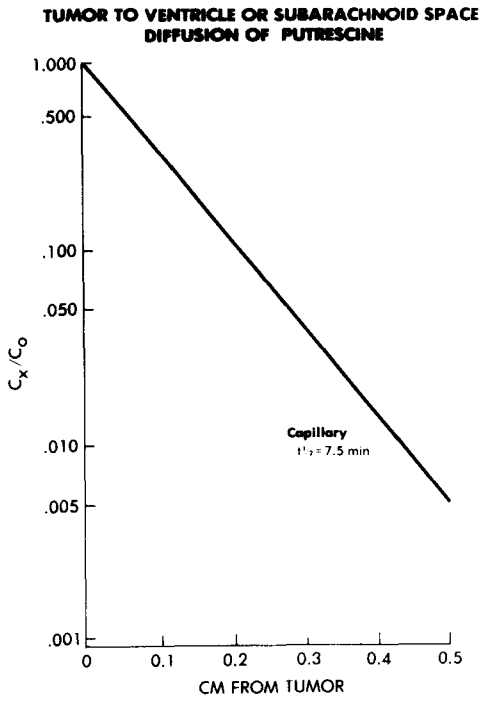

Fig. 3. Theoretical C_x/C_0 plot for Pu in brain. The computations approximate diffusion from a 2 cm diameter spherical tumor outward for a diffusion coefficient of Pu = 1.3×10^{-5} cm^2/sec, which is 40% of D_W (3.3×10^{-5} cm^2/sec; unpublished observations, Dr. Thomas James, using Fourier-transformed NMR), and that Pu crosses brain capillaries with a $t_{1/2}$ of 7.5 min (where $t_{1/2} = \ln 2/K_i$, K_i from equation 2). The transcapillary exchange rates were based on the permeability coefficients computed in this study (Table 1). The algorithm for these computations was derived by Patlak.

*D_W for urea in 2% agar = 1.5×10^{-5} cm^2/sec [13].

†D_W for Pu = 3.3×10^{-5} cm^2/sec. Determined with Fourier-transformed NMR by Dr. Thomas James, Magnetic Resonance Laboratory, Department of Pharmaceutical Chemistry, University of California, San Francisco, California, U.S.A.

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REFERENCES

1. R. A. Campbell, D. R. Morris, D. Bartos, G. D. Daves and F. Bartos, *Advances in Polyamine Research* (2 vols). Raven Press, New York (1978).
2. J. Jänne, H. Pösö and A. Raina, Polyamines in rapid growth and cancer. *Biochem. biophys. Acta (Amst.)* **473,** 241 (1978).
3. H. G. Williams-Ashman and Z. N. Canellakis, Polyamines in mammalian biology and medicine. *Perspect. biol. Med.* **22,** 421 (1979)
4. L. J. Marton, M. S. Edwards, V. A. Levin, W. P. Lubich and C. B. Wilson, Predictive value of CSF polyamines in medulloblastoma. *Cancer Res.* **39,** 993 (1979).

5. J. SEIDENFELD and L. J. MARTON, Biochemical markers of central nervous system tumors measured in cerebrospinal fluid and their potential use in diagnosis and patient management: A review. *J. nat. Cancer Inst.* **63,** 919 (1979).
6. L. J. MARTON, M. S. EDWARDS, V. A. LEVIN, W. P. LUBICH and C. B. WILSON, CSF polyamines: A new and important means of monitoring medulloblastoma. *Cancer (Philad.)*, in press.
7. D. S. FULTON, V. A. LEVIN, W. P. LUBICH, C. B. WILSON and L. J. MARTON, CSF polyamines in patients with glioblastoma multiforme and anaplastic astrocytoma. *Cancer Res.* **40,** 3293 (1980).
8. R. G. BLASBERG, C. S. PATLAK and J. D. FENSTERMACHER, Intrathecal chemotherapy: Brain tissue profiles after ventriculocisternal perfusion. *J. Pharmacol. exp. Ther.* **195,** 73 (1975).
9. J. D. FENSTERMACHER, R. P. RALL, C. S. PATLAK and V. A. LEVIN, Ventriculocisternal perfusion as a technique for analysis of brain capillary permeability and extracellular transport. In *Alfred Benzon Symposium on Capillary Permeability*, p. 483. Munksgaard, Copenhagen (1969).
10. V. A. LEVIN, J. D. FENSTERMACHER and C. S. PATLAK, Sucrose and inulin space measurements of cerebral cortex in four mammalian species. *Amer. J. Physiol.* **219,** 1528 (1970).
11. V. A. LEVIN, H. D. LANDAHL and M. A. FREEMAN-DOVE, 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.* **4,** 499 (1976).
12. J. SEIDENFELD, V. A. LEVIN, W. N. DEVOR and L. J. MARTON, Kinetics and distribution of tritiated putrescine in the domestic cat. *Europ. J. Cancer* **15,** 1319 (1979).
13. C. S. PATLAK and J. D. FENSTERMACHER, Measurements of dog blood–brain barrier transfer constant by ventriculocisternal perfusion. *Amer. J. Physiol.* **229,** 877 (1975).
14. V. A. LEVIN, M. S. EDWARDS and A. BYRD, Quantitative observations of the acute effects of X-irradiation on brain capillary permeability. *Int. J. Radiat. Oncol.* **5,** 1627 (1979).
15. V. A. LEVIN, unpublished observations (1980).