Transport of Cisplatin in Rat Brain Following Microinfusion: an Analysis

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Abstract □ The post-microinfusion transport of *cis*-diamminedichloroplatinum(II) (cisplatin) in rat brain has been modeled as a linear diffusion–reaction–permeation process. The model has been used to analyze the experimental data of Kroin and Penn to obtain the macromolecular binding constant of cisplatin in the brain, $k = 0.0050 \pm 0.0023$ min^{-1}, and the capillary permeability, $p = (9.0 \pm 4.4) \times 10^{-7}$ cm/s. Inclusion of saturation effects led to the same p value and a higher k value of 0.007 min^{-1}. The corresponding diffusion length is 0.8 mm. The reaction constant is similar to those reported for plasma (0.008 min^{-1}) and muscle (0.004 min^{-1}), and the permeability value is within the range predicted by correlation with the permeability–octanol/water partition coefficient. Fits to data were accomplished with mathematical expressions giving the average total platinum concentration in saggital cerebellar sections which were not subdivided. Both time-dependent and steady-state solutions were obtained for the transport model, the former predicting a half-time to steady state of 3 h. Boundary effects were also investigated. Concentration profiles, calculated for a point source and for a 23-gauge cannula, were shown to differ by 7%. Similar comparisons between two profiles, one computed for an infinite diffusion range and another computed for drug diffusion into a flowing cerebrospinal fluid (CSF) at a finite range of 3 mm, showed differences of <3%. Free and bound drug forms, protein turnover, and CSF uptake have been accounted for as well as the percent infusate recoveries at 100 and 160 h reported by Kroin and Penn. The magnitude of convection effects has been assessed by incorporating an upper bound estimate of radial flow into the transport model. The numerical solution led to poorer fitting, but not greatly different, concentration profiles than when convection was ignored. The k and p parameter estimates each dropped by 30%, an upper bound measure of the effect of convection.

In order to predict the local concentration of drug following regionalized or systemic dosing, parameters must be available that account for the transport and reaction of the drug in tissue. In this paper, an analysis is presented of the transport processes associated with the microinfusion of cisplatin in the brain. It is then used to derive the relevant transport parameters from the data of Kroin and Penn.[1]

In microinfusion, a cannula is placed in a tissue and drug reaches the surrounding area primarily by diffusion away from the cannula tip. In the process, a spherical treatment volume is generated whose outer limit is determined by the minimum effective drug concentration. This approach has been applied to murine tumor therapy by Penn et al.,[2] and to studies of feline cortical neuronal plasticity by Kasamatsu, Itakura, and Jonsson.[3]

Transport through the treatment volume may be described by the distributed model of Patlak and Fenstermacher,[4] in which substances move by diffusion and convection across a uniformly distributed capillary bed. This model has been used successfully to describe molecular transport through brain tissue,[5] to investigate drug delivery to the CSF and brain tissue,[6] and to redefine the nature of the peritoneal membrane and transport across it.[7–10] Collins et al.[11] have used the distributed model to account for the concentration-dependent disappearance of 5-fluorouracil from the peritoneal cavity of the rat via the inclusion of a saturable reaction term. Levin, Patlak, and Landahl[12] have also used the distributed model to partially assess the nature and magnitude of the effects on drug delivery of different tumor and normal tissue permeabilities and blood–brain barrier breakdown.

In this paper, we have analyzed the transport of cisplatin following microinfusion into the rat cerebellum, and we have obtained estimates for the primary brain cisplatin transport parameters, the tissue diffusion constant, the capillary permeability, and the irreversible macromolecular binding constant. We have also assessed the relative roles of transient and steady-state transport dynamics, the importance of accounting for finite distribution volumes and saturable binding, and the importance of convection.

We have analyzed the post-microinfusion concentration data profile of total platinum in the rat cerebellum as measured by Kroin and Penn.[1] Their experimental procedure consisted of stereotactically placing a 23-gauge cannula in the center of the cerebellum of a normal rat (Fig. 1), and then infusing cisplatin into the brain at a concentration of 1000 ng/μL (0.9% NaCl, 1% mannitol, and HCl for pH 4.0) by means of an Alzet osmotic minipump at a rate of 0.9 μL/h. After 160 h, the cerebellum was removed, frozen on a slide on dry ice, and cut into sagittal sections of 1-mm thickness. The average total platinum per section was then determined by atomic absorption, yielding the profile tabulated in Table I (from Fig. 1 of Kroin and Penn[1]). The recovery of infused platinum in the cerebellum was also determined as 26.4% at 160 h (27.4% at 100 h). The variable z is the distance from the tip of the infusion cannula to the center of saggital sections cut across the cerebellum. Cisplatin is known to bind essentially irreversibly to various macromolecular components of tissue;[13] therefore, the experimental platinum concentrations represent both free and bound drug. The original data included both right and left concentration profiles that were each nearly log linear. Accordingly, the infusion site ($z = 0$) was determined as the intersection of the two best-fit lines through the two profiles, and all data were then combined into the single profile summarized in Table I. The masses of

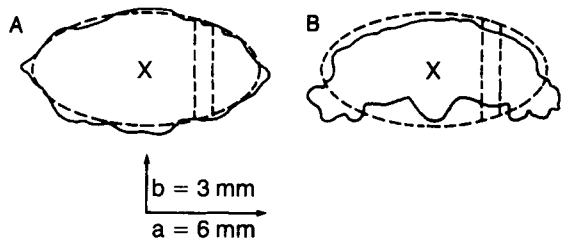

Figure 1—*The rat cerebellum showing its approximate elliptical shape. The infusion site (×) and typical saggital section (---) were analyzed by Kroin and Penn. Key: (A) dorsal view; (B) anterior-posterior view.*

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Table I—Total Platinum Concentrations and Masses of Saggital Sections of the Rat Cerebellum (160-h Infusion)

Distance (z, cm)	Concentration (C, μg Pt/g)	Section Mass	
		Exp. (m, g)	Calc. [$m(z)$, g]
0.03	460	0.038	0.031
0.07	131	0.032	0.030
0.13	77	0.031	0.029
0.17	63	0.017	0.028
0.23	17.2	0.027	0.026
0.27	28.2	0.021	0.024
0.33	8.8	0.017	0.021
0.37	12.8	0.016	0.019
0.43	3.5	0.027	0.015

the sagittal sections, m, were kindly provided by Kroin and Penn, and are also included in Table I; the thickness of these sections varied somewhat with distance, thus accounting for some of the mass fluctuation. An estimate of section mass based upon a prolate spheroid model of the cerebellum, $m(z)$, and will be discussed below.

Theoretical Section

General Considerations—The physical model that we have used to describe cisplatin microinfusion views the transport of the drug through the tissue as a convective and diffusive process near the cannula tip which gives way to a diffusion-reaction process as the drug spreads radially outward from the tip. Cisplatin, like hydroxyurea,[5] is assumed to move primarily through the extracellular space with rapid equilibration between it and the intracellular space. The mathematical model is based on the earlier work of Patlak and Fenstermacher,[4] Levin, Patlak, and Landahl,[12] and Collins and Dedrick,[6] but modified to account for spherical geometry and irreversible chemical reaction.

The platinum-containing species are considered to exist in two pools, one free and one bound. The free pool consists primarily of parent cisplatin, and its monoaquated derivatives. We neglect the small molecular weight adducts with nucleophiles such as cysteine and methionine on the basis that such adducts represent a small fraction of the total platinum at the long infusion times employed by Kroin and Penn.[1] Combining the parent and aquated species poses no problem in the transport sense because both species have nearly identical diffusion constants due to their similar molecular weights and negligible charge difference after screening by the high physiological salt concentration. The bound pool consists of the reaction products of the free pool with macromolecules, particularly sulfur-containing proteins.[13,14]

Accordingly, the general transport equation is:

$$\frac{\partial C}{\partial t} = D\frac{1}{r^2}\frac{\partial}{\partial r}r^2\frac{\partial C}{\partial r} - v_r\frac{\partial C}{\partial r} - pA(C - C_B) - S \quad (1)$$

where C is the average concentration of free drug in the tissue, D is the average diffusion constant in the tissue (equal to the product of the extracellular fraction and tortuosity-corrected extracellular diffusion constant), v_r is the radial velocity, p is the capillary permeability, A is the capillary area per unit tissue volume, S is the average reaction rate of free drug with macromolecules in the tissue, and C_B is the concentration of free drug in the plasma. This equation states that the rate of change of free cisplatin concentration in an infinitesimal volume is equal to the net diffusive flow into it (first right-hand term) plus the net bulk flow into it (second right-hand term) less drug loss across the capillary walls (third term) less drug bound to the macromolecules (fourth term). The radial velocity results from the convective flow of diluent from the cannula and diminishes rapidly with increasing distance from the tip.

The magnitude of the reaction term S is determined by the rate of reaction of both cisplatin and the monoaquo derivative with macromolecules.[13] When the protein nucleophile pool is not substantially depleted and the monoaquated species is at steady state, S may be shown to be linearly dependent on the free cisplatin pool, i.e., $S = kC$. The value of k may be interpreted as the sum of the rate constants for aquation and direct nucleophilic attack on proteins.[15] When saturation of the binding sites occurs, as it does near the tip of the cannula in the experiment of Kroin and Penn,[1] S takes on a nonlinear dependence on C. However, this effect is small for our purposes and we leave its analysis to the *Discussion* section.

Linear Diffusion-Reaction Model of Free Cisplatin—The remainder of this *Theoretical Section* is devoted to an analysis of the linearized version of eq. 1. It is obtained from the general transport equation by making four approximations: (*a*) $C_B = 0$, i.e., reverse transport of free drug from the plasma to brain may be ignored; (*b*) $v_r = 0$, i.e., convection effects may be ignored; (*c*) $\partial C/\partial t = 0$, i.e., the free drug profile is at its steady-state limit over the infusion period; (*d*) $S = kC$ over most of the infusion volume. With these approximations, eq. 1 becomes:

$$\frac{\partial C}{\partial t} = D\frac{1}{r^2}\frac{\partial}{\partial r}r^2\frac{\partial C}{\partial r} - KC = 0 \quad (2)$$

where $K = pA + k$. The first three of the above approximations are justified in *Appendix I*, while the last is justified in the *Discussion* section.

The steady-state solution of eq. 2 can be obtained by observing that it is a modified Bessel equation and applying the appropriate boundary conditions, a finite solution at infinity, and the diffusive flux relation:

$$q = -4\pi\zeta^2 D\frac{\partial C}{\partial r}\bigg|_{\zeta} \quad (3)$$

where q is the mass infusion rate (μmol/s) and $\zeta = .032$ cm is the outer radius of the cannula. The solution is:

$$C(r) = \frac{q}{4\pi rD}\exp(-\delta r)\frac{\exp(\delta\zeta)}{\delta\zeta + 1} = \frac{Q\exp(-\delta r)}{r} \quad (4)$$

where $\delta = \sqrt{K/D}$. This result differs from the $\zeta = 0$ point source solution by only the $\exp(\delta\zeta)/(\delta\zeta + 1)$ factor, a 7% difference when $\zeta = 0.032$ cm and $\delta = 12.6$ cm^{-1}.

Free and Bound Drug and Protein Turnover—The experimental data of Kroin and Penn that we must fit with our model give total platinum concentrations in brain tissue. This concentration not only includes the platinum contribution from free cisplatin (eq. 4) but also the contributions from cisplatin irreversibly bound to macromolecules and from platinum-containing degradation products of these macromolecules. Thus, the total amount of drug present at the end of infusion, $M_T(t)$, is given by:

$$M_T(t) = \int_V C(r,t)dV + \int_V P(r,t)dV + \int_V C_d(r,t)dV \quad (5)$$

where $C(r,t)$ is the free cisplatin concentration, $P(r,t)$ is the platinated-macromolecular concentration, $C_d(r,t)$ is the concentration of platinum-containing degradation products, V is the infusion volume, and t is the infusion time.

The mass of macromolecular-bound platinum is determined by protein turnover dynamics since protein is the quantitatively dominant tissue macromolecule, and its sulfur-containing residues, along with some histidine residues, are thought to be the major binding sites.[13,14] Protein–cisplatin binding is given by:

$$\frac{dP(r,t)}{dt} = kC(r,t) - k'P(r,t) \quad (6)$$

where $P(r,t)$ is the platinum–protein concentration in cisplatin equivalents. Because the free cisplatin concentration reaches steady state early in the infusion, the time-dependence of $C(r,t)$ may be dropped and the differential equation integrated to yield:

$$P(r,t) = (k/k')C(r)(1 - e^{-k't}) \quad (7)$$

where $C(r)$ is given by eq. 4 and k' is related to the protein turnover rate. The time-dependence of $P(r,t)$ must be retained since the characteristic time of protein turnover in the brain (~6 d)[16] is of the same order as the infusion period. The concentration of the degradation product $C_d(r,t)$ is more difficult to obtain, but an approximate description can be made that indicates it is a small contributor to $M_T(t)$ (See *Appendix II*).

We thus drop C_d from further consideration and leave $M_T(t)$ as:

$$M_T(t) = \int_V C(r)dV + \int_V P(r,t)dV \quad (8)$$

Finally, dividing the free cisplatin mass by the total mass, one obtains the free drug fraction:

$$F = \frac{1}{M_T(t)}\int_V C(r)dV = \frac{1}{1 + ktf} \quad (9)$$

where:

$$f = (1 - e^{-k't})/k't$$

Average Platinum Concentration in Sagittal Sections—To obtain an analytical expression for the average platinum concentration in a cerebellar sagittal section, the actual form of the data obtained by Kroin and Penn,[1] we approximated the rat cerebellum as a prolate spheroid with a minor axis b of 3.0 mm and a major axis a of 6.0 mm. If a superposition of this spheroid on the cerebellum is viewed dorsally, the approximation is good. If viewed in the anterior–posterior direction, the spheroid underestimates the volume somewhat in the central region and does not account for the shape of the outlying paraflocculi. However the error in the central region is small, and no data are used from the region of the brain near the paraflocculi.

The equation for the volume of a disk-shaped section cut across the major axis of a prolate spheroid (ellipsoid) at center distance z from the infusion site is:

$$V(z) = \pi\frac{b^2}{a^2}(a^2 - z^2)\Delta z \quad (10)$$

where Δz is the thickness of the section (0.1 cm). Integrating this expression over the sampled cerebellar volume and multiplying by the brain density of ρ = 1.05 g/mL,[17] led to a total tissue mass that agreed with the experimental data. Mass values for constant thickness sections, $m(z) = V(z)\rho$, are recorded in Table I and show reasonable agreement with the experimental data, although there is a trend toward slightly underestimating section masses near the center of the cerebellum and slightly overestimating sections in the outlying regions.

The mass of drug in a tissue section was determined as the concentration integral over the section volume. This is most easily evaluated by changing from spherical polar to cylindrical coordinates. Thus, the free drug section mass, $M(z)$, is:

$$M(z) = \int_0^{R_u}\int_0^{2\pi} C(R,z)\,R\,d\theta dR\Delta z \quad z \geq y \quad (11)$$

where $R^2 = x^2 + y^2$, $r^2 = R^2 + z^2$, R_u is the upper cylindrical radius at a value of z given by $R_u = (b/a)\sqrt{a^2 - z^2}$ and $C(R,z) = C(r)$ of eq. 4. Setting $\alpha^2 = R^2 + z^2$, substitution of eq. 4 into $M(z)$ yields:

$$M(z) = 2\pi Q\int\frac{e^{-\delta\alpha}}{\alpha}\alpha d\alpha\Delta z = \frac{2\pi Q}{\delta}\Big\{\exp(-\delta z) - \exp[-\delta\sqrt{b^2 + [1 - (b^2/a^2)]z^2}]\Big\}\Delta z \quad (12)$$

The average total platinum concentration in the section, $c_p(z)$, is therefore the ratio of the drug mass, eq. 12, to volume, eq. 10:

$$C_p(z) = \frac{WM(z)}{FV(z)} = \frac{2QW}{F\delta}\left(\frac{a}{b}\right)^2 \times \frac{\exp(-\delta z) - \exp\left(-\delta\sqrt{b^2 + [1 - (b^2/a^2)]z^2}\right)}{a^2 - z^2} \quad (13)$$

where W, the atomic weight of platinum, expresses the concentration in platinum equivalents, and F (eq. 9) converts it from a free to a total drug basis. All parameters in this expression are known except δ and the reaction constants k and k' upon which F depends.

Equation 13 is not quite the correct form to be fit to the Kroin and Penn data[1] because these researchers also measured the fraction of infused-drug mass recovered in the cerebellum at the end of the infusion. This recovery fraction, R, was found to be 0.264 at t = 160 h and 0.274 at t = 100 h with little variance.[1] Hence, the concentration expression of eq. 13 must be constrained to reflect this recovery. We will show that this leads to a simple replacement of terms in the leading multiplier of eq. 13. The recovery fraction may be expressed as:

$$R = \left(\frac{1}{qt}\right)\frac{1}{F}\int_\zeta C(r)4\pi r^2 dr \quad (14)$$

where qt is the total amount of cisplatin infused over time t, and the $F^{-1}\times$ integral term is the free plus bound amount of cisplatin recovered in the cerebellum at time t. Using $C(r)$ from eq. 4, this reduces to:

$$R = \frac{1}{tF\delta^2 D} \quad (15)$$

The recovery constraint can then be imposed on eq. 13 by solving eq. 15 for F and substituting the result into eq. 13 to give:

$$C_p(z) = \frac{qWRTt\delta}{2\pi} \frac{e^{\delta\zeta}}{(\delta\zeta + 1)}\left(\frac{a}{b}\right)^2 \times$$

$$\frac{\exp(-\delta z) - \exp\left(-\delta\sqrt{b^2 + [1 - (b^2/a^2)]z^2}\right)}{a^2 - z^2} \quad (16)$$

This is the final form of the nonconvective concentration expression to be fit to the Kroin and Penn data.[1] Note that the expression depends on only one unknown parameter, δ.

Expressions for Reaction Rate Constants and Capillary Permeability—While the fit of $C_p(z)$ to data will yield only the single parameter δ, the additional knowledge of the recovery fractions allows us to derive from it the three unknown parameters of the reaction constant, k, the capillary permeability, p, and the protein turnover constant, k' (via f), given a good independent estimate of the tissue-diffusion constant.

If eq. 9 is substituted into eq. 15 for F, yielding:

$$k = \frac{1}{f}\left(R\delta^2 D - \frac{1}{t}\right) \quad (17)$$

k and k' may be obtained by evaluating this expression at the two infusion times for which recovery data are available and solving the resulting two simultaneous equations for the parameters.

An expression for p can be derived by substituting the definition of δ, eq. 4, into the definition of K, eq. 2, and solving for p. Hence:

$$p = \frac{\delta^2 D - k}{A} \quad (18)$$

As will be seen, eqs. 17 and 18 will provide good k and p estimates for the Kroin and Penn data.[1] The analytic forms of eqs. 15–18 are particularly useful in that they allow much easier statistical fitting to experimental data than would be possible if only numerical solutions were available.

Results

In this section we present our principal quantitative findings based on the theoretical framework developed in the previous section. We first present the parameter values appropriate to cisplatin and the conditions of the Kroin and Penn experiment.[1] Then, we present the fits of theory to experimental data and report the transport and reaction rate constants along with an analysis of their attendant error.

Parameters—The input parameters used in this work are summarized in Table II. The values for q, q_v, R, t, and ζ are taken directly from Kroin and Penn.[1] The values for a and b come from fits to cerebellar mass data as discussed previously. Parameter A has been taken from Rapoport et al.[18]

The diffusivity of cisplatin in brain tissue was assumed to be equal to that of creatinine. This assumption is based on the observation that the rates of absorption of both compounds from the peritoneal cavity of the rat are similar (24.6% compared with 23.1% from 50 mL of normal saline in 1 h).[19,20] This assumption, is supported by our observation that the hydrodynamic radius of cisplatin (crudely based on the crystallographic data of Milburn and Truter[21] and the assumption of free rotation) is similar to the 3.1 Å value reported by Colton et al.[22] for creatinine. The cisplatin tissue diffusion constant in Table II was therefore taken as the creatinine value reported by Patlak and Fenstermacher[4] for the brain.

Fit to Data—Equation 16 was fit to the concentration data of Table I using the P3R nonlinear-regression routine of the BMDP package[23] with least-squares minimization using reciprocal-square concentration weighting (i.e., local variance weighting under the assumption of a constant coefficient of variation.) All input numbers were from Table II. The best fit value of δ was found to be 12.55 ± 1.31 cm^{-1} (Table III, column 2). The fit to the data is shown as the solid line in Fig. 2 where the log concentration is plotted against distance from the cannula tip. The residuals for this fit are evenly distributed around zero.

A χ^2 measure of the goodness of fit was obtained as follows. Each experimental concentration $C_{pe}(z_i)$ was converted to a standard, normal-distribution variate according to:

$$Z_i = \frac{C_{pe}(z_i) - C_p(z_i)}{\sigma(z_i)} = \frac{C_{pe}(z_i) - C_p(z_i)}{\epsilon C_p(z_i)} \quad (19)$$

where $C_p(z)$ is the mean estimate of eq. 16. The second equality follows from the assumption of a constant coefficient of variation over the z range. The Z range was divided into four regions, the numbers of experimental Z_i observations belonging to each region were determined, and the expected number of observations was calculated from corresponding integrals of the standard, normal-distribution function. The χ^2 statistic with the Yate correction applied for one degree of freedom was then calculated. The value of ϵ, needed to obtain numerical Z values, was obtained by constraining the sample estimate of the variance of Z [dependent on ϵ through eq. 19 and distinct from $\sigma(z)$] to equal its standard value of unity. The value for χ^2 was thus found to be 0.47 and is easily significant at the 5% level ($\chi^2 = 3.84$, d.f. = 1, $\alpha = 0.05$).

Sensitivity of the fit to some parameters was investigated. The fit was most sensitive to the value chosen for the recovery R, and relatively insensitive to the ellipse axis lengths. However, because Kroin and Penn reported several values of recovery with little variance[1] (within 0.5%), we did not let R vary in our final fit.

The reaction constants and capillary permeability were obtained from the best fit δ and eqs. 17 and 18: k = 0.0050 min^{-1} and p = 9.0×10^{-7} cm/s, and k' = 1.4×10^{-5} min^{-1}

Table II—Input Parameter Values

Symbol	Definition	Value
q	Mass infusion rate, (μmol/s)	8.3×10^{-7}
q_v	Volume infusion rate, mL/s	2.5×10^{-7}
W	Atomic weight of platinum	195
R	Recovery of cisplatin infusate (at 160 h)	0.264
	(at 100 h)	0.274
t	Infusion time, s	5.76×10^5
ζ	Cannula radius (o.d./2), cm	0.032
a	Major axis of cerebellar ellipsoid, cm	0.6
b	Minor axis of cerebellar ellipsoid cm	0.3
A	Capillary area/unit volume of brain, cm^{-1}	240
D	Cisplatin tissue diffusion constant, cm^2/s	1.9×10^{-6}

Table III—Output Parameter Values

Parameter	Diffusion-Reaction Model	Convection–Diffusion Reaction[a]
δ, cm^{-1}	12.55 ± 1.31	10.40
k, min^{-1}	0.0050 ± 0.0023	0.00336
k', min^{-1}	$(1.4 \pm 1.1) \times 10^{-5}$	1.1×10^{-5}
p, cm/s	$(9.0 \pm 4.4) \times 10^{-7}$	6.2×10^{-7}
F, %	2.15 ± .98	3.09

[a] See the *Discussion* section.

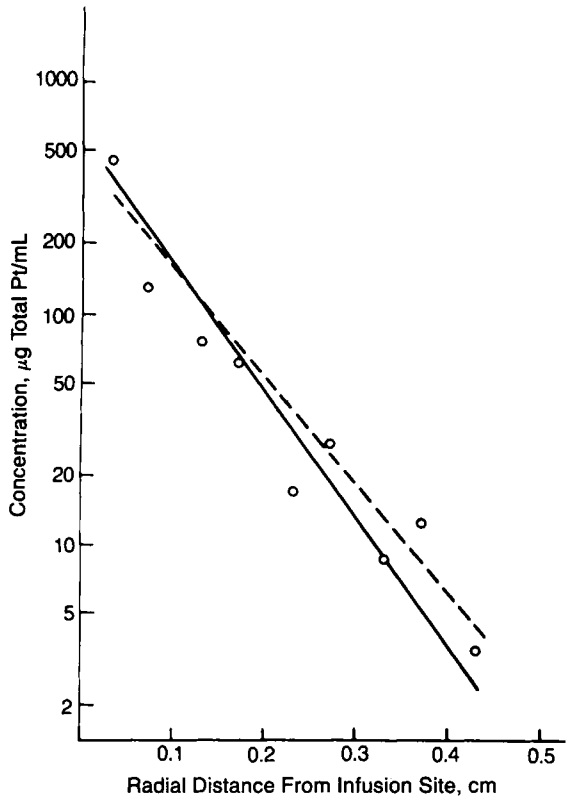

Figure 2—*Log concentration profiles of total platinum concentration versus distance from cannula tip. Key:* (○) *experimental data points of Kroin and Penn; (—) model without convection; (---) model with convection included as upper bound.*

(see Table III, column 2). In addition, the free drug fraction at 160-h postinfusion was calculated from δ and eq. 15 as 2.15% of the total drug-derived platinum.

Error Analysis—The degree of uncertainty in our parameter values was estimated and reported in Table III as the SD of the mean. The SD of δ, σ_δ = 1.31 cm^{-1}, follows directly from the BMDP fitting procedure and its associated estimation of the large sample asymptotic variance.[23] The SDs of k, p, F, and k' were estimated from eqs. 15, 17, and 18 by propagating the errors (variances) of δ, D, R, and A through these equations. The variance of R was estimated as 0.004 from the three recovery values at 160 h reported by Kroin and Penn.[1] The variance of D could only be crudely estimated; we assumed that the diffusion constant was known to within ~50% of its true value, and thus took $\sigma_{\ln D}$ = ln 1.5 = 0.405. The value for σ_A was estimated from Crone[24] as 40 cm^{-1}, the major source of error from this work being the uncertainty in estimated capillary length per cubic millimeter of rat brain. The largest contributor to the SDs of k, p, and F is the uncertainty in the cisplatin diffusion constant. However, even if this diffusion constant were known to perfectly equal its value in Table II (1.9×10^{-6} cm^2/s), the SD's would only drop by 50%. The value for k' is most strongly affected by the uncertainties in the recovery rates.

Discussion

We have proceeded with our transport analysis under the assumptions that the spherical diffusion volume is unlimited, that convection is negligible, and that no saturation of the protein binding sites occurs (i.e., $S = kC$). We may now more critically evaluate these assumptions by assessing the effect of each on the transport results obtained with the linear diffusion–reaction model.

First, consider the finite range of transport. Diffusion is not infinite, as formalized by our earlier boundary condition of a finite concentration at infinity, but is limited by the brain–CSF interface. Hence to assess the magnitude of its effect on transport, we must alter the boundary condition applied at large r values to reflect flow across this boundary and to compare the resulting profile derived from eq. 2 with the earlier infinite range result of eq. 4.

The altered boundary condition approximates the cerebellum as a sphere and, at the r = s brain surface, describes the diffusive species as meeting a flowing CSF. To the extent that the CSF exists as a thin well-mixed fluid layer over the brain and that mass transfer into this layer is rapid, the diffusive flux at the surface may be regarded at steady state as input to the CSF flowing at Q_F mL/s. Hence, under these conditions, the CSF drug concentration approaches that of free cisplatin at the brain surface, and the appropriate boundary condition is:

$$-4\pi D s^2 \frac{\partial c}{\partial r}\bigg|_{r=s} = Q_F C(s) \qquad (20)$$

When the transport equation, eq. 2, is solved subject to the boundary conditions of eqs. 3 (with $\zeta \to 0$) and 20, we obtain:

$$C(r) = \frac{q}{4\pi D}\left[\frac{\cosh \delta r}{r} + \frac{\sinh \delta r}{r}\,\frac{(\delta r \sinh \delta s + B \cosh \delta s)}{(B \sinh \delta s - \delta s \cosh \delta s)}\right] \qquad (21)$$

where δ is defined in eq. 4 and B is equal to $Q_F/(4\pi D s) - 1$.

The concentration profiles for the finite and infinite range cases, eqs. 4 (with $\zeta = 0$) and 21, are compared in Table IV. For these calculations, $D = 2 \times 10^{-6}$ cm^2/s, $q = 2.5 \times 10^{-4}$ μg of cisplatin/s, $\delta = 12.6$ cm^{-1}, $Q_F = 3.33 \times 10^{-5}$ mL/s[25] and s = 0.3 cm, values characteristic of rat brain tissue and the Kroin and Penn[1] infusion (see the *Results* section). The s value corresponds approximately to the distance from the cannula tip to the nearest major CSF boundary.

It is evident from Table IV that the two concentration profiles are similar. Hence, we concluded that refined descriptions of brain–CSF mass transfer were not required in place of the infinite diffusion–reaction model, since relatively small concentration errors were involved in the tail of the concentration profile.

Next, consider the assumption of negligible convection. It plays no role at large distances but, as discussed in *Appendix I*, it does have an effect within the first half-millimeter of the cannula tip. To be sure that this does not lead to a significantly different fit of the concentration profile to the data, we derived the convective counterparts of the concentration profiles of eqs. 4 and 16 and the parameter relations of eqs. 17 and 18, and redetermined the best fit transport constants

Table IV—Free Cisplatin Concentrations, Infinite versus Finite Regions of Diffusion

r, cm	Infinite C(r), μg/mL	Finite[a] C(r), μg/mL
0.01	877.40	877.40
0.05	106.22	106.23
0.10	28.36	28.37
0.20	4.04	4.06
0.30	0.77	0.80

[a] s = 0.3 cm, where s is the radius of the finite sphere.

numerically (see *Appendix III*). The calculation is an upper bound measure of convective effects because the radial velocity has been overestimated as a result of assuming that the diluent is conserved throughout the cerebellum, in spite of the flow that must occur across capillary walls.

From the best fit of the convection-dependent concentration profile (eq. A7) to the data of Table I, δ was found to be 10.40 cm^{-1} (Table III, column 3), and the fit to the concentration data appears as the dashed line of Fig. 1. The χ^2 statistic of 1.30 (d.f. = 1), calculated as before, shows that the fit is significant at the 5% level but is less good than the diffusion model result where $\chi^2 = 0.47$. The reaction constants and capillary permeability were obtained from δ and eqs. A8 and 18; $k = 0.00336$ min^{-1}, $p = 6.2 \times 10^{-7}$ cm/s, and $k' = 1.1 \times 10^{-5}$ min^{-1} (see Table III, column 3). The free drug fraction was calculated numerically from eq. 14 as 3.09%.

Note that even with convection represented as this upper bound, the reaction rate and capillary permeability constants are not greatly different from the estimates obtained with no convection present. Because the convection-dependent k, k', and p values lie within the first standard error of the corresponding non-convection-dependent values (see Table III), it is apparent that neglect of convection introduces less uncertainty than that associated with concentration measurement error (reflected in δ variance) and D, R, and A errors. Together, these observations allow us to conclude that convection does not play a significant role in transport parameter estimation under microinfusion conditions similar to those employed by Kroin and Penn.

Now consider the possibility that protein binding sites may begin to saturate over the long infusion times encountered here. All other things remaining unchanged, this would mean that the predicted total platinum concentration profile would be lower, particularly in the region near the cannula tip where the probability of reaching saturation is the highest. To assess the effect of this phenomenon on predicted profiles and parameter estimates, we again solved eq. 1 numerically but, this time, ignored convection and employed a nonlinear saturable reaction term, S.

The exact form of S is determined not only by saturation but also by the speciation of cisplatin. At intracellular pH and chloride content, the dominant tissue environment, cisplatin exists both as a parent compound and a monoaquated form.[26] Other derivatives are also present but, except at extreme saturation, they can probably be neglected on the grounds that strong cellular nucleophiles will react with the monoaquo species before it undergoes much further reaction. Both parent cisplatin and the monoaquated species react with nucleophiles, the latter at an overall reaction rate close to the rate of aquation of cisplatin.[13] The direct cisplatin–nucleophile reaction in brain cells is of similar but somewhat lower value when the nucleophile concentration is taken into account.

Since the overall rates of reaction are similar for both reaction arms, we have approximated the saturable reaction of cisplatin with protein as that of a single pool of parent cisplatin and monoaquated derivative reacting with the sulfur residues of the protein. Accordingly, the reaction term is:

$$S = k''C\langle P_0 - P\rangle$$

where k'' is the pool-average bimolecular reaction rate constant, C is the free cisplatin concentration, P_0 is the protein concentration in cisplatin equivalents (i.e., residue concentration), and $\langle P_0 - P\rangle$ is the unbound protein concentration averaged over the infusion time. (See *Appendix IV* for a discussion of the time-averaging and derivation of $\langle P_0 - P\rangle$ and its use in eq. 1). Obviously if the protein were not saturated, the reaction term would become $S = k''CP_0$ and one could identify the linear reaction k (eq. 2) with $k''P_0$. On the other hand, as saturation occurs, P approaches P_0 in value, and the reaction slows.

Solving eq. 1 with the nonlinear reaction S included, followed by use of the solution in a new section-average concentration expression (see *Appendix IV*), allowed us to again fit the data of Table I and obtain a best estimate for k''. As before, the recovery was constrained to be 0.264 after 160 h of infusion. The value of P_0 was the only new input parameter, and we took its value to be 7 mM based on the combined cysteine, methionine, and to some extent, histidine content of brain tissue.[14,27,28] The total protein methionine and cysteine concentration in brain is 12.8 mM and the histidine concentration is 23mM. From X-ray studies of platinum (II) chloride binding with various proteins,[15] we estimated that only 25% of the sulfur residues are exposed to cisplatin attack. Hence we estimated the effective methionine plus cysteine concentration as 3.2 mM. We also estimated histidine to be about 1/15 as active as methionine in reacting with cisplatin,[14] yielding an effective histidine nucleophile concentration of 1.5 mM. Adding these effective concentrations together yielded 4.7 mM. The final P_0 value of 7 mM was obtained by adding to it the rat brain concentration of glutathione,[29] since it is not mobile across cell membranes and has a 3-d half-life approaching that of proteins.[30]

The best k'' rate constant was found to be 1.0×10^{-3} $min^{-1} \cdot mM^{-1}$, corresponding to a linear reaction rate of $k = k''P_0$ of 0.0071 min^{-1}. This value is somewhat larger than the 0.005 min^{-1} linear estimate in Table III, but still lies within the same range. The corresponding concentration-distance profile is virtually identical to the solid line in Fig. 2 except for a decrease below $z = 0.07$ cm; the concentration estimate for the $z = 0.03$ cm section (which includes the cannula tip) is lower by 25% at 300 μg of platinum per milliliter.

The best estimate of capillary permeability is not sensitive to the choice of P_0 and remains as 9.0×10^{-7} cm/s. The sensitivity of k'' to the choice of P_0 was assessed by again estimating its value for $P_0 = 1$ mM. The value of k'' was found to rise to 0.023 $min^{-1} \cdot mM^{-1}$, corresponding to a linear k of the same value. However, the fit of the concentration profile to the experimental data is poorer than when $P_0 = 7$ mM. The χ^2 value increased from 2.7 to 4.2, and the profile is quite flat at 150 μg of cisplatin per milliliter before resuming log-linear behavior beyond $z = 0.2$ cm.

We conclude that saturation of sulfur-containing residues does play a role in determining drug–protein reaction rates, principally by lowering the total platinum concentration estimate of the central cerebellar section. The effect is relatively small; however, the linear result underestimates the saturation result by only 30%.

In view of the spherical geometry of diffusion and the elliptical volume sections that determine the functional form of the section-average platinum concentration (eq. 16), it was not apparent at the outset of our analysis that the distributed model could reproduce the log-linear concentration profile reported experimentally. However, inspection of the tissue-section mass integral of eq. 12 reveals that it accounts for this behavior, at least at low z values where the bracketed term limits to $\exp(-\delta z)$. Because this behavior is also present in the concentration expression of eq. 16 (the $a^2 - z^2$ volume term is essentially independent of z values up to 0.2 cm), the log-linear concentration profile is explained at low z values. This result also shows that an initial estimate of δ may be made from the slope of the log-linear profile ($\delta \sim 13$ cm^{-1}).

Furthermore, with rearrangement of the right-hand side of eq. 16 to yield the form $f(z)\exp(-\delta z)$, evaluation shows that the $f(z)$ multiplier of $\exp(-\delta z)$ differs from unity by <3% at $z = b$ and for some distance beyond. Hence, the log-linear

concentration profile is explained over the 0.5-cm data range of Kroin and Penn.[1]

In summary, our analysis has shown that the transport of cisplatin, following microinfusion of the drug to the center of the cerebellum of the rat, can be described by unbounded spherical diffusion accompanied by largely linear macromolecular reaction and loss across capillary walls. Neglect in this model of the limited diffusion range imposed by the existence of the CSF–brain boundary leads to an insignificant error in the predicted concentration profiles. Likewise, the finite geometry of the 23-gauge cannula also exerts a small effect, leading to only a 7% increase in the concentration profile relative to that computed for a point source.

Our best linear estimate for the rate constant describing the reaction of cisplatin with macromolecules in brain tissue is 0.0050 ± 0.0023 min^{-1}. Inclusion of convection effects would decrease this value (but by no more than 30%), while inclusion of saturation effects would increase it (by ~40%). This rate constant is in the same range as that measured for the biotransformation of cisplatin to fixed metabolite in plasma, 0.0082 min^{-1}, and that reported for other tissues based on pharmacokinetic modeling, i.e., 0.0041 min^{-1} for muscle to 0.0902 min^{-1} for kidney.[31]

Our best estimate for capillary permeability in the brain is $(9.0 \pm 4.4) \times 10^{-7}$ cm/s. This value may be compared to that expected from the permeability–octanol/water partition coefficient correlation of Rapoport et al.[18] Hoeschele et al.[32] reported a 1-octanol/saline partition coefficient for cisplatin of 7.3×10^{-3} at 37°C. The Rapoport et al. correlation[18] predicts a permeability of 7.1×10^{-7} cm/s, a figure lying within the standard error band of our estimate.

The results of this work show that the transport of cisplatin through brain may be described in the same distributed model framework used previously to describe the flow of CaEDTA, hydroxyurea, 5-fluorouracil and other chemicals through tissue.[5,7,11] In addition, methods have been presented which will allow ready assessment of the importance of transient kinetics, limited diffusion ranges, saturation, and CSF uptake in future models. The parameters that we have estimated will allow the prediction of platinum concentrations in normal brain tissue following infusion, and, if combined with suitable dose-response relations, may allow regions of cell killing to be estimated. Similar work needs to be carried out on tumor tissues, particularly in view of its often greater permeability,[12] in order to assess the capacity of the drug for tumor killing without undue toxicity.

Appendix I

Approximations Yielding Linear Diffusion-Reaction Model—*Neglect of plasma to brain tissue backflow*—The value of C_B may be shown to be negligibly small relative to C by estimating the value of C_B at 160 h (the infusion time of Kroin and Penn[1]) from a simple one-compartment model of cisplatin pharmacokinetics. For constant infusion, this model takes the form:

$$\frac{V dC_B}{dt} = q - \beta C_B$$

where V is the volume of distribution (245 mL in the rat), β is the total body clearance, and q is the infusion rate (0.9 μg of cisplatin per hour in the Kroin and Penn experiment[1]). The β corresponds to a half-life of 22 min in the rat.[33] The pharmacokinetic model with $C_B(0) = 0$ predicts that C_B at 160 h would be 0.002 μg of free cisplatin per milliliter. By contrast, Kroin and Penn[1] measured a total equivalent cisplatin concentration in tissue at 160 h of ≥3 μg/g within 5 mm of the injection site. If the free fraction is 2% (<10% is suggested by the plasma binding studies of Patton et al.,[34] and 2% is obtained later from our infusion model), $C(160\text{ h}) = 0.064$ μg of free cisplatin per milliliter. Thus, $C > C_B$ by a factor of 32 or more over the rat cerebellum, justifying the dropping of this term.

Neglect of Convection—The validity of neglecting the convection (ν_r) term is more difficult to show. However, we can estimate an upper bound of the convection effect by assuming that the drug diluent does not cross the capillary walls and is, therefore, conserved as it flows toward the periphery of the cerebellum. In this case, the convective to diffusive flux ratio is:

$$\frac{j_B}{j_D} = \frac{\nu_r C}{-D(dC/dr)} \tag{A1}$$

where:

$$\nu_r = \frac{q_v}{4\pi r^2} \tag{A2}$$

and q_v is the volume flow rate of drug solution (0.9 μL/h) and C has been approximated by the steady-state diffusion-reaction solution of eq. 1, with $C_B = 0$ (see eq. 3). If $(k + pA)/D \sim 160$ cm^{-2} (as can be estimated from the Kroin and Penn profile) and $D \sim 2 \times 10^{-6}$ cm^2/s, then the j_B/j_D flux ratio ranges from 0.22 at r = 0.032 cm (corresponding to the o.d. of the 23-gauge cannula used by Kroin and Penn[1]) to 0.12 at r = 0.05 cm and 0.04 at 0.1 cm. Thus we may conclude that convection plays a significant role within the first half-millimeter but declines in importance beyond this distance. Therefore, to first-order accuracy, convection may be ignored. In later discussions, we include the convection term and directly assess the error introduced by this approximation.

Applicability of Steady State—The time-dependent solution of eq. 2 for a point source can be obtained by noting that the substitution of $C = C_1 \exp(-Kt)$ converts it to a pure diffusion equation in C_1. The solution for C_1 for a continuous point source (infusion source) has been given by Carslaw and Jaeger[35] and, when combined with the exponential expression for C, gives:

$$C(r,t) = \frac{q}{8(\pi D)^{3/2}} \int_0^t \exp\left[\frac{-r^2}{4D(t-t')}\right] \times \exp\left[-K(t-t')\right] \frac{dt}{(t-t')^{3/2}}$$

where q is the mass infusion rate (μmol/s). Reevaluation in special functions gives:

$$C(r,t) = \frac{q}{16\pi f d^{3/2}} \left[\exp(2fg)\operatorname{erfc}\left(\frac{f}{\sqrt{t}} + g\sqrt{t}\right) + \exp(-2fg)\operatorname{erfc}\left(\frac{f}{\sqrt{t}} - g\sqrt{t}\right)\right] \tag{A3}$$

where $f = r/\sqrt{4D}$ and $g = \sqrt{K} = \sqrt{pA + k}$. This solution may be used to estimate the time required to approach steady state. Of the two erfc terms, only the second is important at long times. Half-time to steady state may be estimated by the time required for this erfc term to reach its mid-range value of 3/2. At a point 4-mm distant from the infusion site, near the cerebellar boundary, this time is 3 h for the parameters above. Because this time is much less than the 160-h duration of the cisplatin infusion, we concluded that only steady state solutions were required for further analysis.

Appendix II

Contribution of Protein Degradation Products to Total Platinum Profile—If the degradation product is defined as the small platinum-bearing breakdown product pool that leaves the cell (the larger intermediate products are indistinguishable in a transport sense from the original protein adducts), then its transport is approximately described by:

$$\frac{\partial C_d}{\partial t} = D_d\nabla^2 C_d - \lambda C_d + k'P(r,t)$$

$$C_d(r,0) = 0,\ C_d \text{ bounded} \qquad \text{(A4)}$$

where D_d is the degradation product diffusion constant, λ describes transport across the blood–brain barrier, and $P(r,t)$ is the platinum–protein concentration of eq. 7. This equation may be solved by a superposition of decaying time-dependent spherical shell solutions[35] chosen to describe the $k'P(r,t)$ source. While a complete time-dependent $C_d(r,t)$ may be obtained in this way, we derived only the infinite time limit since this overestimated C_d yet was sufficient to show that it could be neglected. The result is:

$$C_d(r) = \frac{kq}{8\pi Dr\sqrt{\lambda D_d}\,(\delta\zeta + 1)}\left\{\left(\frac{1}{\delta_d - \delta}\right)\times\right.$$

$$\left.\left[e^{-\delta(r-\zeta)} - e^{-\delta_d(r-\zeta)}\right] + \frac{1}{(\delta + \delta_d)}\left[e^{-\delta(r-\zeta)} - e^{-\delta_d(r+\zeta)}\right]\right\}$$

where $\delta_d = \sqrt{(-k' + \lambda)/D_d}$ and $\delta = \sqrt{(k + pA)/D}$, as above. If we let $\zeta = 0$ for simplicity, then the ratio of the degradation product concentration to the free cisplatin concentration (eq. 4) is:

$$\frac{C_d(r)}{C(r)} = \frac{k}{\sqrt{\lambda - k')D_d}}\,\frac{\delta_d}{(\delta^2_d - \delta^2)}\left[1 - e^{(\delta - \delta_d)r}\right] \qquad \text{(A5)}$$

This expression shows that the degradation product becomes a higher percentage of the mobile platinum as r increases ($\delta > \delta_d$ for any reasonable selection of parameters). Its numerical value is 1.14 at r = 0.4 cm if the transport constants D_d and λ are set equal to those of cisplatin (D and pA), $k' = 1.34 \times 10^{-6}$ s^{-1} (protein $t_{1/2}$ = 6.0 d),[16] $k = 0.005$ min^{-1}, and $pA = 2 \times 10^{-4}$ s^{-1} (initial estimates of k and pA from Farris et al.[31] and Rapoport et al.[18]).

Since the protein-bound platinum will be found to be ~45 times the free cisplatin concentration (see the *Results* section), the $M_T(t)$ expression of eq. 8 and the 1.14 C_d/C ratio indicates that the degradation product is only 3% of the total platinum concentration at r = 0.4 cm. Even if the degradation diffusion constant were halved and the transport constant were reduced to one-fifth, the degradation product would be only ~20% of the total platinum at r = 0.4 cm and considerably less at smaller r values.

Appendix III

Convective-Diffusive Transport of the Drug—The convective counterpart of eq. 4 begins with eq. 1. As before, only the steady-state limit need be considered and C_B may be ignored. The reaction term S is kept linear with the same form as in eq. 2. The velocity v_r was represented by the upper bound expression (see eq. A2 of *Appendix I*) rather than a more rigorous expression because we are only interested at this time in estimating whether the flow effect makes a significant difference in the pure diffusion-reaction result of eq. 4. Hence, the convection–diffusion reaction case is described by:

$$0 = \frac{1}{r^2}\frac{\partial}{\partial r}r^2\frac{\partial C}{\partial r} - \frac{v_r}{D}\frac{\partial C}{\partial r} - \delta^2 C \qquad \text{(A6)}$$

where eq. 1 was initially divided by D and δ^2 was substituted for $(pA + k)/D$.

The boundary conditions are finite concentration at infinity and the combined diffusion-convection flux condition at the cannula tip where r = ζ, i.e.:

$$q = 4\pi\zeta^2\left(v_r C_0 - D\frac{\partial C}{\partial r}\right)_{r=\zeta}$$

At $\zeta = 0.032$ cm neither diffusion nor convection is dominant. The cisplatin concentration (C_0) in the infusate equals q/q_v.

Analytic solutions of this equation for our parameter values are not easily obtained and, hence, it must be solved by numerical methods. We used finite difference methods involving central difference representation of the derivative and the Taylor expansion approximation of the third boundary condition as outlined.[36] Two hundred grid points were used.

The section-average platinum concentration consistent with a numerical $C(r)$ may be derived from the first equality of eq. 13 after substitution of eq. 10 for $V(z)$, eq. 11 for $M(z)$, and eq. 14 for F to yield:

$$C_p(z) = \frac{qWRt}{2\pi}\left(\frac{a}{b}\right)^2\frac{\int_0^{R_u} C(R,z)RdR}{(a^2 - z^2)\int_\zeta^\infty C(r)r^2dr} \qquad z \geq \zeta \qquad \text{(A7)}$$

where R_u is defined below eq. 11, as are the relations between r, R, and z. The integrals may be obtained by Simpson integration of the numerical $C(r)$ function obtained from eq. A6. Note that because $C(r)$ only depends on the unknown δ (see eq. A6), $C_p(z)$ also depends on this single unknown.

The associated expression for reaction constants may be derived from eqs. 9 and 14 as:

$$k = \frac{1}{f}\left(\frac{Rq}{4\pi\int_\zeta^\infty C(r)r^2dr} - \frac{1}{t}\right) \qquad \text{(A8)}$$

The permeability constant is again given by eq. 18, except that k is represented by eq. A8 rather than eq. 17.

Appendix IV

Saturable Transport—The approximate equation for saturable transport is eq. 1, with $v_r = 0$, $C_B = 0$, and $S = k''C(P_0 - P)$ where P is the bound protein concentration in cisplatin equivalents. Because the time of approach to saturation is much greater than the time required to achieve a diffusion–permeation balance, we may still assume that $\partial C/\partial t \sim 0$ throughout the tissue volume. Hence:

$$0 = D\frac{1}{r^2}\frac{\partial}{\partial r}r^2\frac{\partial C}{\partial r} - pAC - k''C(P_0 - P) \qquad \text{(A9)}$$

The P of this equation is time dependent, reflecting the degree of saturation reached at any time. However, the corresponding pseudo-steady-state C is much more weakly dependent on time. This follows from our linear analysis

where it was shown that pA is about twice $k''P_0$ (= k) and thus the profile is dominated by the time-independent pA coefficient.

Time averaging eq. A9 leads to:

$$0 = D\frac{1}{r^2}\frac{\partial}{\partial r}r^2\frac{\partial <C>}{\partial r} - pA<C> - k''<C><P_0 - P> \quad \text{(A10)}$$

where the weak time dependence of C allows us to rewrite $<C(P_0 - P)>$ in the form shown. This is our basic equation for saturable free cisplatin transport, one in which $<C> \sim C$ and the reaction term may be regarded as the rate of free cisplatin binding with the time-averaged unbound protein concentration. $<P_0 - P>$ may be approximated from the counterpart of eq. 6:

$$\frac{dP}{dt} = k''C(P_0 - P) - k'P \qquad P(0) = 0 \quad \text{(A11)}$$

as:

$$<P_0 - P> = P_0\, w(C) = P_0\left[1 - \frac{k''C}{E}\left(1 + \frac{e^{-Et} - 1}{Et}\right)\right] \quad \text{(A12)}$$

where t is the infusion time, $E = k''\,C + k'$, and the time dependence of C has been neglected.

The section average concentration, $C_p(z)$, was computed directly from eqs. 8 and 10 as:

$$C_p(z) = WM_T(t)/V(z) = \frac{W}{V(z)}\left[\int CdV + \int PdV\right]$$

where eq. 11 is substituted for the first integral and the solution of eq. A11 for the second integral. The result is:

$$C_p(z) = \frac{2W\int_0^{R_u} C(R,z)\,[1 + k''P_0 tf\, w(C)]RdR}{\left(\frac{b}{a}\right)^2\left(a^2 - z^2\right)} \quad \text{(A13)}$$

where $C(R,z) = C(r)$ was obtained from numerical integration of eq. A10 (see *Appendix III*) and the other functions were defined previously. Equation A13 was fit to the data of Table I, numerically searching for the k'' and p values that best fit the data subject to the recovery constraint at 160 h. The value of k' was not altered from its earlier value.

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