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Concentration-Dependent Disappearance of Fluorouracil from Peritoneal Fluid in the Rat: Experimental Observations and Distributed Modeling

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Abstract □ The rate of disappearance of fluorouracil from peritoneal fluid has been experimentally measured and mathematically modeled. The experimental data were obtained following the instillation of 50 ml of dialysis fluid which contained an initial fluorouracil concentration ranging from 24 μM to 12 mM. The rate of disappearance was strongly dependent upon concentration. A distributed model has been formulated which incorporates concepts of diffusion with saturable metabolism and nonsaturable capillary uptake in the tissue surrounding the peritoneal fluid. This model successfully describes the experimental observations and also suggests that the effective penetration depth into tissue is highly dependent upon concentration.

Keyphrases □ Fluorouracil—concentration-dependent disappearance from peritoneal fluids, rats □ Pharmacokinetics—concentration-dependent disappearance of fluorouracil from peritoneal fluid, rats □ Peritoneal fluid—concentration-dependent disappearance of fluorouracil, rats

Although intraperitoneal injections are extensively used for the administration of drugs to rodents, there are few studies which examine the kinetic features of this route (1, 2). When drugs are administered in a small volume, it is usually assumed that absorption occurs through pathways which lead to the portal vein. For larger volumes, as used in peritoneal dialysis, nonportal pathways such as the ventral abdominal wall, diaphragm, and retroperitoneal tissues may have a significant role. Lymphatic uptake, although largely unexplored, is assumed to be quantitatively unimportant for substances with small molecular weights.

The rate of disappearance of drugs from peritoneal fluid is primarily a consequence of the concentration gradient established between peritoneal fluid and surrounding tissue. As drug molecules diffuse into this tissue, they can be carried away by capillary blood, metabolized by enzymes in the tissue, or be bound to tissue constituents. Removal by capillary blood is kinetically a first-order process, while metabolism and tissue uptake or binding are potentially saturable since these processes depend upon a finite number of sites.

The intraperitoneal administration of the pyrimidine analog, fluorouracil, is currently undergoing clinical evaluation for the treatment of several cancers which are initially confined to the abdomen (2, 3). Since the clinical range of intraperitoneal fluorouracil concentration is restricted by therapeutic considerations, a previously developed rat model was used for these peritoneal disappearance studies. The metabolism of fluorouracil is saturable (4), and at least some tissues surrounding the peritoneal cavity are sites for this metabolism (5). No tissue binding has been reported.

Data have been collected on the rate of peritoneal disappearance over a wide range of initial concentrations in order to observe metabolism in both linear and nonlinear regions. A distributed model has been formulated which incorporates concepts of diffusion with chemical reaction and capillary uptake in the surrounding tissue. The nonlinear partial differential equation was solved numerically with the appropriate initial and boundary conditions. The solution suggests that the effective penetration depth into the tissue and the rate of removal from the peritoneal cavity are concentration dependent.

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EXPERIMENTAL

Separation of Fluorouracil from Its Metabolites—Fluorouracil is extensively metabolized by both anabolic and catabolic enzymes. The goal of this separation procedure was to isolate the parent compound, fluorouracil, but not necessarily to identify individual metabolites. A 10–25-μl sample of peritoneal fluid was applied to 250 μm of silica gel G TLC plates[1]. Each plate included a [^{14}C]fluorouracil[2] standard. The plates were developed for 10 cm by the upper phase of the solvent system of Koechlin *et al.* (6): ethyl acetate (60%, v/v), formic acid (5% v/v), and water (35%, v/v). Samples were counted in a liquid scintillation counter with automatic external standardization. In this system, 97% of [^{14}C]-fluorouracil radioactivity was located above R_f 0.45, while all metabolites were below R_f 0.45 ([^{14}C]floxuridine[2], 98%; [^{14}C]urea[3], 99%; [^{14}]fluoro-ureido-propionic acid, 99%). Since dihydrofluorouracil is unstable in this system, it was measured as fluoro-ureido-propionic acid or urea (6). [^{14}C]Fluoro-ureido-propionic acid was isolated by the procedure of Chauduri *et al.* (7) from the urine of rats given [^{14}C]fluorouracil. Urine was placed on anion exchange resin[4] (10 × 1 cm) and eluted with 50 ml of water, 80 ml of 0.05 *M* formic acid, 150 ml of 1.5 *M* formic acid, and finally 50 ml of 0.5 *M* HCl. [^{14}C]Fluoro-ureido-propionic acid was found in the third fraction. [^{14}C]Fluorouracil standard eluted in the second fraction, as validated by TLC.

Experimental Procedures—Female Sprague-Dawley rats[5] (220–240 g) were anesthetized with 50 mg/kg ip pentobarbital. Supplemental doses of 15 mg/kg im were given as required. Fluorouracil[2] was dissolved in peritoneal dialysis solution[6] (major components: 1.5% dextrose; 132 mEq Na; 3.5 mEq Ca; 1.5 mEq Mg; 99 mEq Cl; 35 mEq lactate), and the solution was warmed to 37°. [^{14}C]Fluorouracil was added to yield total fluorouracil concentrations ranging from 24 μM to 12 m*M*. Fifty milliliters of drug solution was injected into the peritoneal space through an 18-G needle. Periodic 100-μl samples were obtained by transcutaneous puncture with a 23-G needle. Rectal temperature was maintained at 37 ± 0.2° by means of a heat lamp and temperature controller[7].

Simulations—Although an analytical solution is not feasible for the model presented in the Appendix, Eqs. 1 and 2 can be integrated numerically, using Eqs. 3*a*, 3*b*, and 3*c* as initial and boundary conditions. The simulations presented in this paper were computed by means of the implicit form of the finite difference method, based on the algorithm of Carnahan *et al.* (8).

RESULTS

Measurements of fluorouracil disappearance from peritoneal fluid are plotted in Fig. 1. Initial fluorouracil concentrations in the peritoneal fluid ranged from 24 μM to 12 m*M*. There is a very strong dependence of disappearance rate on concentration. The rate of disappearance at low concentration is 10-fold greater than at high concentration.

The simulated disappearance curves shown in Fig. 1 were obtained by numerical integration of Eqs. 1–3 from Appendix I. The model parameters are discussed in Appendix II. The *a priori* parameters associated with the simulations in Fig. 1 provide an adequate representation of the data over the five orders of magnitude that drug concentration was measured. Although a statistical best fit was not attempted[8], an example of the ability of the distributed model to match more closely the experimental data is presented in Fig. 2. For these simulations, V_{max} was arbitrarily adjusted to 36 nmole/min/g and K_M to 5 μM, while the other parameters were unchanged.

Solution of Eqs. 1–3 also yields information about the concentration profile of drug in tissue. There are no experimental data for comparison, and these calculations are highly dependent on assumptions about model geometry. Nonetheless, there are some important predictions worthy of consideration. Figure 3 shows that the tissue concentration profile requires 15 min to become fully developed for an initial peritoneal concentration of 12 m*M*. In contrast, Fig. 4 shows that only 1 min is required for profile development when the initial peritoneal concentration is 24 μM. At high concentrations, >600 μm are needed for the tissue concentration to drop to 5% of the tissue fluid interfacial value, while <200 μm are required for the same percentage drop at low concentration. Also, there are qualitative differences in the shape of the profiles. At low concentrations, the fully developed profile is log-linear, since metabolism dominates and is essentially linear in this concentration range. At high concentration, the profile is convex downward as a result of changes in the extent of saturation of metabolism.

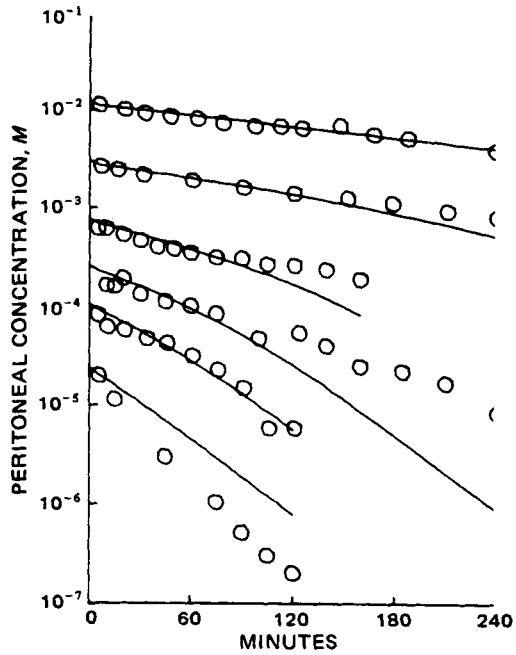

Figure 1—*Disappearance of fluorouracil from peritoneal fluid. Key: experimental data (O) plus simulations (—). Simulation parameters presented in Appendix II.*

DISCUSSION

The strong concentration-dependence of fluorouracil disappearance from peritoneal fluid is a novel observation and a challenge for pharmacokinetic modeling. It is likely that the peritoneal disappearance is

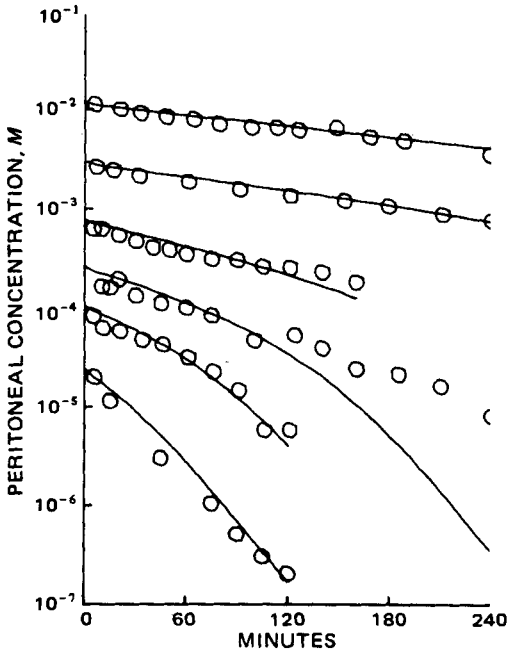

Figure 2—*Disappearance of fluorouracil from peritoneal fluid. Key: experimental data (O) plus simulations (—). Simulation parameters are the same as in Fig. 1, except that* K_M = 5 μM *and* V_{max} = 36 *nmole/min/g.*

[1] Analtech, Newark, Del.
[2] Obtained from the Developmental Therapeutics Program, National Cancer Institute.
[3] New England Nuclear, Boston, Mass.
[4] Dowex-1 anion exchange resin, BioRad, Richmond, Cal.
[5] Taconic Farms, Germantown, N.Y.
[6] Inpersol, Abbott Labs, North Chicago, Ill.
[7] Yellow Springs Instrument Co., Yellow Springs, Ohio.
[8] Such calculations would have limited usefulness in the absence of experimental data for tissue concentration profiles. These calculations would also be extremely expensive.

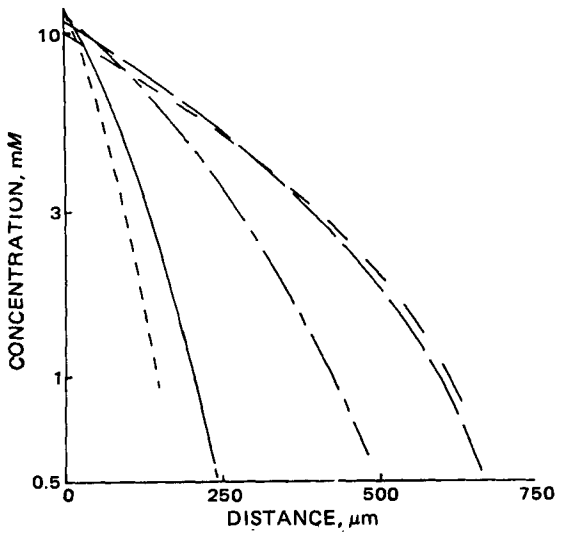

Figure 3—*Simulated time course for development of concentration profile in tissue. Initial concentration at interface is 12 m*M. *Key: (- - -) 30 seconds after dialysis fluid is added; (——) 1 min after; (— –) 5 min; (— —) 15 min; and (– – –) 30 min.*

a consequence of diffusion of drug into the surrounding tissue, where removal of fluorouracil by blood and metabolism by tissue enzymes are competing parallel processes. Although the drug is stable in peritoneal fluid itself, it is quite plausible that cells in the peritoneal membrane, and especially in the surrounding tissues, have sufficient enzyme capacity to account for the observed increase in fluorouracil disappearance at low concentrations. Both catabolic and anabolic enzymes for fluorouracil metabolism are widespread in body tissues, including abdominal organs (7, 9).

The selection of a modeling approach must be justified by the nature of the information obtained from the model. In the analysis of fluorouracil peritoneal disappearance data, there are two major items of interest: the depth to which the drug can penetrate into tissue and the relative role of removal by blood flow compared with metabolic elimination. Compartmental analysis is the major tool used in pharmacokinetic analysis. This approach consolidates areas of the body into regions in which the concentation is a function of time but not of anatomic position. Compartmental analysis cannot provide assistance for the two items outlined previously. Therefore, the next level of pharmacokinetic modeling has been utilized for the analysis of peritoneal uptake data, namely, direct consideration of positional variations in concentration.

Penetration distance is the crucial parameter for two major clinical questions which need to be answered regarding intraperitoneal therapy:

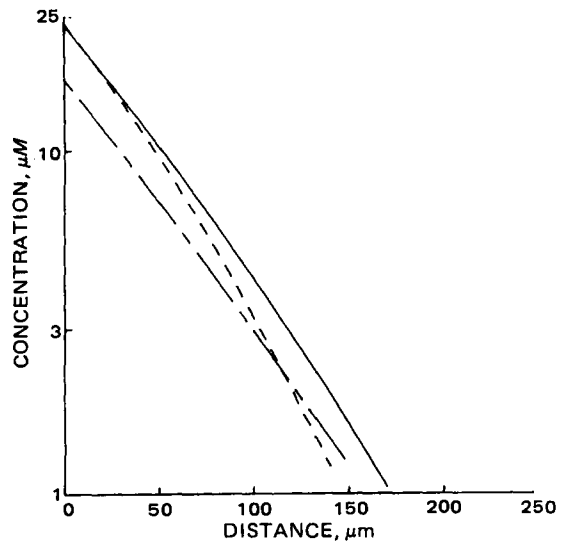

Figure 4—*Simulated time course for development of concentration profile in tissue. Initial concentration at interface is 24 μ*M. *Key: (- - -) 30 sec; (——) 1 min; (— –) 5 min after dialysis fluid is added.*

(a) How large a tumor will be exposed to drug levels which are in excess of those which can be achieved with systemic therapy. (b) Since GI mucosal cells are a major site for drug toxicity, what concentration can be administered safely, *i.e.*, does not cause penetration of high drug levels to these cells.

For comparative purposes, a penetration depth may be defined as the distance at which the tissue concentration has declined to some percentage of the interfacial concentration. As noted previously, the 5% penetration depth is a strong function of concentration: <200 μm for an interfacial concentration of 24 μM and >600 μm for 12 mM. Thus, not only are tissues exposed to higher concentrations as peritoneal fluid concentration is raised, but progressively deeper tissues are exposed. When referred to a typical cell diameter of 10 μm, these penetration distances are equivalent to a depth of 20–60 cells.

The second major question about peritoneal uptake concerns the relative roles for blood and metabolic removal. Fortunately, this question can be addressed without the specification of a particular geometry for tissue uptake. As long as the ratio of blood removal capacity to metabolic capacity is independent of position, some conclusions can be reached about their relative roles, regardless of the exact three-dimensional location of these processes in tissue. The dominant process is simply determined by a comparison of the removal terms in Eq. 2, $V_{max}/(K_M + C)$ *versus* PS/V. For the parameters given in Appendix II, the two removal processes are equally effective when C = 2.4 mM. At the highest concentration used in this study, 12 mM, blood flow removal is 5-fold greater than metabolic removal. However, as fluorouracil penetrates the tissue, its concentration is reduced until the dominant role is shifted to metabolism. In the linear range of metabolism ($K_M \ll C$) the rate of metabolism is 79 times as great as the rate of removal by flowing blood. The specific shape of the tissue concentration profile is dependent on model geometry, but the trends illustrated in Figs. 2 and 3 are generalized properties of distributed modeling.

Distributed models can be very useful in pharmacokinetics in special circumstances such as when there is a need for understanding the positional variation of concentration. Construction of distributed models requires information about kinetic properties as a function of position, and verification of distributed models requires experimental determination of concentration profiles in tissue. This combination of experimental data and distributed modeling is very rare. One excellent study describes the penetration of several anticancer drugs into brain tissue (10). However, there is no study in which a metabolized substance has been quantified. Indeed, the technology for exposing tissue to a rapidly metabolized drug and then quenching the metabolism and assaying for spatial variation in the concentration of the parent within ~200 μm probably does not exist.

Since the profile data are not available for fluorouracil, all quantitative aspects of the proposed model cannot be validated. The principal goal of this modeling effort has been to demonstrate the plausibility of a distributed model for the analysis of pharmacokinetic data. The model structure has been shown to be consistent with the peritoneal disappearance data, but no attempt has been made to choose the best model parameters based on statistical criteria. Despite its limitations, the distributed modeling process has given insight into the mechanisms of disappearance for fluorouracil and has suggested a strong dependence of penetration depth on peritoneal fluid concentration. Further experimental design could be guided by these results.

APPENDIX I

Distributed Model for Peritoneal Disappearance—The rate of disappearance of fluorouracil from peritoneal fluid can be governed by a variety of resistances to mass transport. The model which is described in this section is based on diffusion of fluorouracil through the extracellular space of peritoneal tissue. As will be discussed, there is no transcellular pathway, but intracellular drug is in equilibrium with extracellular concentration.

For a constant peritoneal fluid volume (V_{ip}), the rate of change of drug concentration in the peritoneal fluid (C_{ip}) depends on the diffusivity of the drug in surrounding tissue (D), the area available for transport (A), and the concentration gradient in the tissue at the tissue–fluid interface. The concentration in the surrounding tissue (C, extracellular or diffusible concentration) depends on both anatomic position and time. The diffusion problem has been represented by a one-dimensional model in which constant kinetic properties are assumed throughout the tissue; *i.e.*, diffusivity, capillary permeability (PS/V), maximum metabolic capacity (V_{max}), and half-saturating concentration (K_M). R is the ratio of intracellular to extracellular drug mass.

$$V_{ip}\frac{dC_{ip}}{dt} = -DA\left.\frac{\delta C(x,t)}{\delta x}\right|_{x=0} \quad \text{(Eq. 1)}$$

$$\frac{\delta C}{\delta t} = \frac{D}{R+1}\frac{\delta^2 C(x,t)}{\delta x^2} - \frac{1}{(R+1)}\frac{PS}{V}C(x,t) - \frac{1}{(R+1)}\frac{V_{max}C(x,t)}{K_M + C(x,t)} \quad \text{(Eq. 2)}$$

The term PS/V should be interpreted as effective capillary permeability, since tissue perfusion rate may also limit the rate of capillary removal. Metabolism is referred to extracellular fluorouracil concentration, since that was the basis in the hepatocyte parameter estimation (11).

Initially, the tissue concentration is zero throughout, and there is always some point ($x = x'$) where the gradient has vanished. As will be discussed, resistance to mass transfer posed by the peritoneal fluid is assumed to be negligible. Thus, the initial and boundary conditions are:

$$C_{ip}(0) = C(0,0) = C_0 \quad \text{(Eq. 3a)}$$

$$C(x,0) = 0 \quad \text{(Eq. 3b)}$$

$$\frac{\delta C(x',t)}{\delta x} = 0 \quad \text{(Eq. 3c)}$$

Resistance Within Peritoneal Fluid—The peritoneal fluid drug concentration, which is measured experimentally, is the bulk fluid concentration. The concentration at the interface between peritoneal fluid and tissue will be less than the bulk fluid concentration if the rate of drug transport in fluid is not much faster than the rate capacity of the tissue to take up drug. Resistance to drug transfer in the bulk fluid might be visualized as a stagnant layer adjacent to the tissue. Calculations for intraperitoneal fluorouracil based on stagnant layer thickness reported previously (12) suggest that this resistance is negligible at high concentrations and has a mild influence at low concentrations.

Diffusion Pathways—Drug diffusion through tissue may involve transcellular pathways in addition to extracellular movement. Several possibilities exist: (*a*) extracellular drug movement and very little, if any, intracellular uptake; (*b*) very rapid transcellular movement so that drug effectively moves through both pathways; and (*c*) moderate transcellular movement so that drug moves only extracellularly but equilibrates with intracellular space. The third option was selected based on a comparison of transfer rates through extracellular space, across cells, and across capillaries. A half-time for cellular uptake of fluorouracil of 40 sec or less can be calculated from reports for hepatoma cells (13), Ehrlich ascites cells (14), and L 1210 cells (15). Since the transcapillary exchange half-time of 140 sec, calculated from PS/V, is substantially slower, there is sufficient time for intracellular exchange and option (*a*) can be eliminated. The characteristic time (x^2/D) for extracellular diffusion is only 0.2 sec, based on a half-cell diffusion distance (5 μm) and a fluorouracil diffusivity of 1.2×10^{-6} cm^2/sec. Therefore, option (*b*) was eliminated since extracellular movement is very rapid compared to transcellular movement.

APPENDIX II

Parameter Selection—There are seven parameters in Eqs. 1 and 2. Since V_{ip} was experimentally set to 50 ml, six parameter values must be estimated:

1. Michaelis constant (K_M) was fixed at 30 μM. This value has been reported for fluorouracil metabolism in rat liver cells (11).
2. Maximum metabolic capacity per cubic centimeter of tissue (V_{max}) was fixed at 71 nmole/min/cm^3, a value which has been reported for rat liver tissue (11). Values of V_{max} or K_M for other tissues have not been reported.
3. Area of tissue available for flux (A) was set at 125 cm^2. If the measurements of Esperanca and Collins (16) of peritoneal surface area in human infants and adults are extrapolated using the 0.7 power of body weight to a 200-g rat, ~125 cm^2 is obtained.
4. Diffusivity (D) in tissue was estimated by the method of Schultz and Armstrong (12). First, aqueous diffusivity was estimated to be 10×10^{-6} cm^2/sec, based upon its molecular weight (17). Then the aqueous diffusivity was corrected for extracellular fluid fraction (0.3) and tortuosity (2.5) to yield a value of 1.2×10^{-6} cm^2/sec.
5. Capillary removal rate (PS/V) is the product of capillary permeability (P, cm/sec) and capillary surface per volume of tissue (S/V, cm^2/cm^3). Reported values of PS/V range from 0.004 sec^{-1} for the gastric wall of dogs for a hexose (18) to 7.6×10^{-4} sec^{-1} for glucose in muscle capillaries (19). Since 0.001 sec^{-1} is a reported value for tissue perfusion in rat muscle (20), the effective value for PS/V is limited by perfusion. A value of 5×10^{-4} sec^{-1} is consistent with these observations and provides reasonable agreement between the simulations and the data in the high-concentration concentration region where PS/V dominates.
6. The ratio (R) of intracellular to extracellular fluorouracil amount is calculated from the apparent distribution volume for fluorouracil and the extracellular fluid fraction of tissue. Based on a whole body distribution volume of 60% of body weight (4) and an extracellular fluid fraction of 0.3 (see item 4), an equal amount of drug is in the two spaces: $R = 1$.

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