Intratumor Pharmacokinetics, Flow Resistance, and Metabolism during Gemcitabine Infusion in ex Vivo Perfused Human Small Cell Lung Cancer

Paul E. G. Kristjansen, Thomas J. Brown, Lisa A. Shipley, and Rakesh K. Jain
Edwin L. Steele Laboratory, Department of Radiation Oncology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114 [P. E. G. K., R. K. J.], and Lilly Research Laboratories, Indianapolis, Indiana 46285 [T. J. B., L. A. S.]

ABSTRACT

The relationship between tumor physiology and the pharmacokinetics of 2',2' difluorodeoxycytidine [gemcitabine (dFdC)] in ex vivo perfused human small cell lung cancer was examined. Two small cell lung cancer sublines, 54A and 54B, with known in vivo sensitivity to dFdC, were grown as tissue-isolated tumors in athymic mice and perfused ex vivo with or without 20-40 μM dFdC. Arteriovenous differences in gases, pH, and metabolites were determined before and during drug infusion. The geometric flow resistance (FR) of individual tumors was calculated, and dFdC and its inactive metabolite 2',2' difluoro-2-deoxyuridine were determined by high-performance liquid chromatography of consecutive samples from the output line. Both tumors had prominent lactate production concurrent with a significant O2 consumption. The arteriovenous pH drop was ~0.3 in both tumor lines. Significant metabolic differences between 54A and 54B tumors were found that elucidated previously described differences further. Pharmacokinetic analysis showed that the initial tumor uptake of dFdC was flow limited, and a significant inverse correlation between the geometric FR and initial drug uptake was found. The rate constant for recovery of the drug in the tumor outflow was greater in 54B tumors (P < 0.05), and the geometric FR was greater in 54A tumors (P < 0.01). The drug conversion rate was independent of physiological parameters. Attempts to modify the delivery of dFdC should be directed at the tumor blood flow distribution. More generally, our experimental model provides useful new insight into metabolism and intratumor pharmacokinetics of chemotherapeutic agents in solid tumors.

INTRODUCTION

A perfused tumor offers the advantages of control over the physiological, pharmacological, and biochemical composition of the arterial input and free access to the venous output. Such preparations permit studies of substrate turnover and intratumor pharmacokinetics of therapeutic and diagnostic compounds, in addition to assessments of physiological parameters in solid tumors. Recently, we developed an ex vivo perfusable, tissue-isolated human tumor preparation using tumor xenografts in nude mice (1). A tissue-isolated tumor has a single artery and a single vein as its sole vascular connection to the host organism. Our experimental model was based on the original rat tumor preparation by Gullino and Grantham (2) using the left ovarian fat pad as the inoculation site. A different tissue-isolated model, the inguinal or epigastric preparation, also provides excellent access to the venous outflow of tumors, and as such, it has been used extensively (3, 4) in nude rats for metabolic studies in human xenografts. In the epigastric preparation, the arterial input is not isolated from the systemic circulation; therefore, exclusive ex vivo perfusion of the intact solid tumor is more feasible with the ovarian preparation (1, 5). Various tissue-isolated tumor preparations have been applied for studies of blood flow (2-9), O2 and glucose turnover (3, 5, 10-13), and drug transport (14-18).

Our reason to develop the nude mouse preparation was to provide a tool for studies of the kinetics and transport of key metabolites and therapeutic agents in human tumor xenografts. On this background, we now present the first characterization of geometric FR in perfused human tumor xenografts and the relationship of this and other physiological parameters with the uptake of a new cytostatic compound, dFdC. dFdC is a relatively new agent with proven activity in several solid tumors, including lung cancers (19). Two human SCLC tumor lines, 54A and 54B, which have different sensitivities to dFdC (20), have been shown previously to represent different metabolic phenotypes (21), in addition to different physiological properties with regard to the disposition of a radiographic contrast agent (22) and to the accumulation of fluorine derived from dFdC (20). In the present series of 36 perfusion experiments, the mechanisms of these differences between the two SCLC subpopulations are elucidated further.

MATERIALS AND METHODS

Animals. Female 8- to 10-week old Ncr/Sed-nu/nu athymic mice, bred in the Edwin L. Steele Laboratory's animal...
facility, were used. The body weight was in the range of 22–27 g, and the animals were fed sterilized standard laboratory rodent chow and sterilized water ad libitum. Prior to all surgical procedures, the animals were anesthetized with a s.c. injection of ketamine (10 mg/kg) and xylazine (1 mg/kg) in 0.9% NaCl solution. Institutional guidelines for animal welfare and experimental conduct were followed.

**Tumors and Transplantation.** The human SCLC xenografts CPH SCLC 54A and 54B are subpopulations of the same patient tumor. The 54A cells are hypotetraploid, and they contain twice the amount of DNA than the 54B cells (23). The tumor lines are indistinguishable by morphology and growth kinetics, but 54A tumors are more radiosensitive than 54B tumors, and 54B tumors have shown greater sensitivity to dFdC (20). In previous studies, differences in metabolic and physiological characteristics of 54A and 54B tumors *in vivo* have been outlined (20–22). Tumors were grown as tissue-isolated tumors, as described in detail elsewhere recently (1). In brief, a connective tissue pedicle containing the left ovarian vessels was prepared surgically in a female nude mouse and positioned subsequently in a s.c. pouch wrapped in a sterile parafilm bag. Before the bag was sealed, 0.02 ml tumor slurry was injected at the distal end of the pedicle, and in ~4 weeks, tumors grew to sizes of 500–1000 mg. During growth, the bag was replaced every 5–6 days.

**Experimental Design.** Tissue-isolated SCLC tumors of the 54A and 54B types were perfused with a cell-free modified KH perfusate at physiological pH and temperature. Following clearance of blood from the tumor circulation, corresponding values for perfusion pressure (P<sub>TOT</sub>) and flow (Q) were determined in each tumor during stepwise increments of the pump output. The pressure was increased until abnormal dilation of the tumor vein was observed. The pressure drop over the tumor vascular bed (ΔP<sub>TUM</sub>) was calculated as described below. On the basis of the ΔP–Q curves, the total FR, the tumor geometric resistances (Z<sub>T</sub> and Z<sub>0</sub>), and the zero flow intercept (P<sub>0</sub>) were calculated in each individual tumor. Concurrently, the O<sub>2</sub> consumption, CO<sub>2</sub> production, and arteriovenous pH difference were assessed, as well as the glucose consumption and lactate production. Thereafter, the perfusion was shifted to a parallel circuit with an identical perfusate containing dFdC. In this part of the experiment, the pump setting was kept constant at the second highest output level from the initial recordings in each tumor, i.e., the highest pump output with no abnormal dilation of the tumor vein. The tumor effluent was collected continuously with a MacLab computer (AD Instruments, New South Wales, Australia). The perfusate was a cell-free KH solution: 118 mm NaCl; 4.7 mm KCl; 1.2 mm KH<sub>2</sub>PO<sub>4</sub>; 1.2 mm MgSO<sub>4</sub>; 2.55 mm CaCl<sub>2</sub>; 11.1 mm glucose; 7000 units/liter Na-heparin; and 2.5% (w/v; 25 g/liter) BSA (A7030; Sigma Chemical Co., St. Louis, MO). The buffer was adjusted with 2.99 g/liter NaHCO<sub>3</sub> to maintain a pH of 7.4 after equilibration. Oxygenation and pH stabilization were achieved by prototype gas exchangers, in which the perfusate was led through 16 feet of Silastic tubing (Dow-Corning, Midland, MI), while equilibrating with warm humidified 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Prior to each experiment, the two perfusates (with or without the drug) recirculated through gas exchangers for 30 min at 37°C. The perfusates were filtered twice through sterile 0.22-μm filters. During the experiments, the perfusates were kept in a heated bath and led via the pump through the heated and humidified gas exchanger and further via the three-way stopcock into the arterial catheter. The partial pressures of gases and the pH were measured repeatedly using an ABL 300 gas analyzer (Radiometer, Copenhagen, Denmark) in microsamples drawn from the effluent and afferent lines. Q was determined by differential weighing of the collecting tubes, assuming a density of the perfusate of 1.0. At the end of each experiment, the arterial catheter was infused with an Evans blue solution to document the vascular integrity. When no leakage was found, the tumor was excised and unwrapped from the parafilm bag for further checking. Cross-sections were inspected visually to obtain a crude estimate of the amount of hypoperfused or necrotic areas. The tumors were not used if significant leakages were observed or if <75% of the tumor volume turned blue by the dye test. See Fig. 1 for a schematic of the experimental setup.

**Drugs.** dFdC (19) has a molecular weight of ~300 and no significant protein binding. The parent drug and tetrahydouridine were provided by Lilly Research Laboratories. Ther-
apeutic solutions were made by dilution with 0.9% NaCl, and the solution was added to the drug-containing perfusate reservoir to final concentrations of 20–40 μM in the perfusate. This concentration range was chosen because it has been shown that the cellular conversion into the phosphorylated active metabolite dFdCTP is saturable at consistent dFdC concentrations of 20–30 μM in vitro and in vivo in the plasma of patients (24–26).

The drug perfusate was mixed thoroughly by stirring and by the preexperimental recirculation of the perfusate through the gas exchanger and back to the reservoir.

**Drug and Metabolite Assays.** Samples were collected continuously in preweighed vials, which contained 10 μl 12 mg/ml tetrahydrouridine solution. The vials were frozen immediately in liquid N₂ and stored at −80°C until analysis. Concentrations of dFdC and dFdU were determined by high-performance liquid chromatography. Following neutralization, the analytes were separated using a Zorbax-RX column (5 μm) and a mobile phase containing 50 mM ammonium acetate and acetonitrile (93:7). The analytes were observed using UV detection at 254 nm. The assay for dFdC and dFdU was linear in a range of 0.25–40 mg/ml perfusate.

The afferent and efferent concentrations of lactate and glucose were determined with enzymatic assay kits (Sigma) in 200-μl samples drawn repeatedly at 6–8-min intervals.

**Statistical Analysis.** Curve fitting and estimation of parameters, including calculation of uncertainties, correlation coefficients, and the residual, were performed using the MacCurveFit software (Macintosh; Apple Computer Co., Cupertino, CA). Statistical analysis was performed using SPSS software (SPSS, Inc., Chicago, IL). All statistical analyses were nonparametric, because specific stipulations regarding the nature of underlying distributions of the parameters were not justified. For comparison of parameters between 54A and 54B tumors, the two-tailed Mann–Whitney U test was applied. Bivariate correlation between parameters was estimated using the Spearman test, and a partial correlation analysis was performed when separation of the effects of multiple parameters was of interest. In such cases, the specific influence of a single parameter was excluded from the calculation to evaluate the partial influence of the other parameters.

**RESULTS**

Of a total of 36 experiments, 23 were deemed of sufficient quality to be included. In eight experiments, we failed to obtain a sufficient correlation between flow and pressure because of leakage and/or other technical difficulties. In three experiments, the tumor perfusion, evaluated by the Evans blue test, was minimal. In those cases, the ΔPO₂ and ΔPCO₂ were extraordinarily low during perfusion, indicating the presence of excessive shunting. Finally, in two cases, calculation of the fluid loss, based on the baseline pump output, disclosed a fluid recovery of <60%. In those experiments that were included, the median fluid loss, calculated in this way, was 4–5%.

**Pharmacokinetic Analysis.** All concentration-time curves of the parent drug in the outflow reached equilibrium within approximately 15 min (see Fig. 2), indicating that the net accumulation and/or metabolism was saturated at that time point. In all cases, the equilibrium output concentration remained less than the input, indicating that binding or conversion of the parent drug occurred at a constant rate throughout the 45 min of perfusion. The individual curves were fitted using a nonlinear algorithm. In each individual case, the monoexponential function below provided the best fit when compared with a biexponential function. The validity of the fit was determined on the basis of the residual, on the uncertainties of estimated parameters, and on the calculated r² values for the fit. The mass balance for the drug infusion into a tumor volume (V), is:

\[
\frac{dC}{dt} = Q\left(1 - \frac{C}{R}\right) - kVC = Q\left(1 - \frac{C}{R\alpha}\right)
\]

The solution to this equation is:
Fig. 2 Example of a normalized concentration-time curve of the parent drug dFdC and the inactive metabolite dFdU in the tumor effluent of a perfused 54B tumor during continuous infusion with dFdC. Dashed line at top, input drug concentration (100%).

\[ C_{\text{NORM}}(t) = \alpha (1 - e^{-\beta t}) \] (A)

where

\[ R = \frac{Q}{\alpha \beta \nu} \]

and

\[ k = \beta (1 - \alpha) \]

\( C_{\text{NORM}} \) is the output drug concentrations, normalized with regard to the input concentration. The parameter \( \alpha \) contains information about the conversion and \( k \), and \( \beta \) contains informations about the passage of dFdC through the tumor, i.e., the initial distribution phase. When \( k = 0 \), i.e., there is no conversion or binding, \( \alpha = 1 \). When \( r = 1 \) and \( k = 0 \), then \( \beta = Q/V \). Finally when \( R > 1 \), the drug partition in the tumor is greater than in the perfusate. When fitting the drug output data to Equation A, the uncertainties were less than 5% in all cases, whereas they were several orders of magnitude greater with any biexponential function. Correspondingly, the residual in terms of the sum of squared errors was significantly lower with a monoexponential function than with a biexponential function. In all the examined tumors, Equation A produced fits with an \( r^2 \) in the range of 0.93–0.99. The \( \beta \) values were significantly greater in 54B tumors than in 54A tumors \((P = 0.025)\). The other estimated parameters are shown in the middle section of Table 1.

**Tumor Flow Parameters.** \( Q \) (ml/min) is given by (27, 28):

\[ Q = \frac{\Delta p}{FR} = \frac{\Delta p}{\eta Z} \] (B)

where \( \Delta p \) is the pressure drop across the vascular bed, \( \eta \) (g/ml/s = centipoise = 1.22 mm Hg × min × 10^-6) is the viscous resistance, and \( Z \) (ml^-1) is the geometric resistance. This equation can be divided by the tumor weight \((W; \text{g})\), and thus expressed per unit weight:

\[ q = \frac{Q}{W} = \frac{\Delta p}{\eta z} \] (C)

where \( q \) (ml/min/g) is the perfusion rate, and \( z \) (g/ml), i.e., the product of \( Z \) and \( W \), is referred to as the extrinsic geometric resistance. In a perfused organ, the slope of the pressure flow \((\Delta p-Q)\) curve is equal to \(1/FR = \eta Z^{-1}\); therefore, \( Z \) can be determined, because \( \eta \) is known (6). Above a certain perfusion pressure, the \( Z \) (or \( z \)) maintains a constant minimum value, \( Z_0 \) (or \( z_0 \)), corresponding to a linear \( \Delta p-Q \) curve. At lower pressures, the curve departs from linearity with a decreasing slope. The extrapolated abscissa intercept is the \( P_0 \) (see Fig. 3).

The venous outflow occurred at atmospheric pressure; therefore, the perfusion pressure represents the pressure drop across the catheter (and connection unit) and the tumor vascular bed. Baseline curves were obtained routinely to determine the catheter resistance. The baseline curves were always linear in the pressure range of 0–300 mm Hg, and the proportionality factor \( i.e., \) the tube resistance \((R_{TUBE})\) was calculated easily.

The pressure drop across the tumor is:

\[ \Delta p_{TUM} = P_{TOT} - Q \times R_{TUBE} \] (D)

The abscissa intercepts, \( P_0 \), for each individual tumor were the same in \( \Delta p_{TOT-Q} \) compared with \( \Delta p_{TUM-Q} \) plots. The tumor \( \Delta p-Q \) plots were linear above tumor pressures of ~25 mm Hg. No significant difference between \( P_0 \) of 54A and 54B tumors was found. The viscosity of the KH perfusate with 2.5% BSA is 0.9 centipoise (6); thus, the \( Z_0 \) and \( z_0 \) values were calculated for each examined tumor, using Equation B, as shown in Table 1. The tumor extrinsic geometric resistance, \( z_0 \), was significantly greater in 54A tumors than in 54B tumors \((P = 0.0093)\). \( Q \) and \( q \) were both significantly greater in 54B tumors than in 54A tumors, as shown in Table 1. \( Q \) was correlated positively with \( \beta \) \((P = 0.002)\), whereas the correlation of \( \beta \) with \( q \) did not reach conventional levels of statistical significance. In contrast, \( R \) was correlated inversely with \( q \) \((P = 0.001)\) but not with \( Q \). Concordantly, there was an inverse correlation between \( Z_0 \) and \( \beta \) \((P = 0.002)\). \( Z \) and \( Q \) are related intimately (Equation B), and when controlling for the influence of \( Q \), no other parameter came out as being significantly correlated with the initial distribution phase.

The \( Z_0 \) was correlated strongly with the tumor weight \((P = 0.002)\), which is not surprising, because \( z = Z \times W \). The \( Z_0 \) values, on the other hand, did not correlate significantly with \( W \) \((P = 0.5)\). When controlling for the influence of \( W \), partial correlation analysis indicated significant inverse correlations between \( k \) and \( Z_0 \) \((P = 0.022)\) and \( z_0 \) \((P = 0.018)\), whereas \( \beta \) in this case did not show partial correlation with any specific parameter.

**Metabolic Parameters.** The \( O_2 \) consumption rate (ml \( O_2/h/100 \text{g} \)) was calculated using the Fick principle:

\[ V_{O_2} = q \Delta p O_2 \times S \] (E)

using an \( S \) (solubility constant) value of 0.003 ml \( O_2 \times 100 \text{ml}^{-1} \times \text{mm Hg}^{-1}\) (29), adjusted to the inflow temperature using tabular material provided elsewhere (30). Corresponding values...
Table 1  Physiological and pharmacokinetic parameters in 54A and 54B tumors

Numbers are medians; ranges are given in parentheses. Statistical comparison is performed by Mann-Witney’s U test, and the correlation analysis is by the Spearman test.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SCCL 54A</th>
<th>SCCL 54B</th>
<th>54A vs. 54B (P)</th>
<th>Correlation with dFdC disposition (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W (g)</td>
<td>0.69 (0.41–1.24)</td>
<td>0.58 (0.13–1.4)</td>
<td>NS*</td>
<td>β</td>
</tr>
<tr>
<td>P (µl/min)</td>
<td>130 (100–300)</td>
<td>193 (140–330)</td>
<td>0.067</td>
<td>k</td>
</tr>
<tr>
<td>q (µl/min × g)</td>
<td>180 (143–322)</td>
<td>345 (200–643)</td>
<td>0.0095</td>
<td>R</td>
</tr>
<tr>
<td>z0 (10⁴ g × cm⁻³)</td>
<td>15.85 (4.81–30.18)</td>
<td>3.86 (2.14–9.3)</td>
<td>NS</td>
<td>ΔpH</td>
</tr>
<tr>
<td>P0 (mm Hg)</td>
<td>15 (10–33)</td>
<td>21 (8–38)</td>
<td>NS</td>
<td>Δ Lactate (µmol min⁻¹ g⁻¹)</td>
</tr>
<tr>
<td>β (min⁻¹)</td>
<td>0.25 (0.16–0.35)</td>
<td>0.31 (0.28–0.42)</td>
<td>0.0251</td>
<td>Δ Glucose (µmol min⁻¹ g⁻¹)</td>
</tr>
<tr>
<td>α</td>
<td>0.955 (0.88–0.99)</td>
<td>0.955 (0.79–1.0)</td>
<td>NS</td>
<td>Δ O₂ standard (µl O₂ g⁻¹ min⁻¹)</td>
</tr>
<tr>
<td>k (10⁻² min⁻¹)</td>
<td>1.04 (0.49–1.61)</td>
<td>1.15 (0.81–1.57)</td>
<td>NS</td>
<td>ΔO₂ maximal (µl O₂ g⁻¹ min⁻¹)</td>
</tr>
</tbody>
</table>

a NS, not significant; (−), inverse correlation; (+), positive correlation.

**DISCUSSION**

Our data indicate that the tumor uptake of dFdC was flow limited. This conclusion is based primarily on the nonparametric
This difference was present both in conventional s.c. xenografts and in tissue-isolated tumors. In the present study, we demonstrate that the initial distribution phase of another low molecular weight agent, dFdC, is faster in 54B tumors than in 54A tumors. Taken together, these data document the existence of a tumor line-specific difference in disposition kinetics of non-protein-bound, low molecular weight agents between 54A and 54B tumors. The significantly different extrinsic geometric resistance, $z_0$, between tumor lines seems to be a key component. The $Z_0$ and $z_0$ values shown in Table 1 correspond to tumor perfusion pressures, $ΔP_{\text{TUMO}} > 25$ mm Hg; i.e., they are obtained from the linear pressure flow behavior, found in our tumors at pressures above this limit. The range of values is in concert with geometric resistances reported previously for various normal organs and rat tumors (9), as reviewed extensively by Sevick and Jain (6). The $z$ is governed primarily by the vascular morphology of tumors, which has been shown to be qualitatively and quantitatively different from normal tissues in different respects (33), as reviewed elsewhere (27, 34, 35). The prime characteristic is heterogeneity and lack of normal organization, which accounts for temporal and spatial heterogeneity of tumor blood flow as demonstrated in rat tumors (8) and in our SCLC tumor lines (22). In the present study, flow was based exclusively on measurements of the total tumor effluent flow. Therefore, the perfusion rate, $q$, represents an approximation, which is but an average of the actual microcirculatory perfusion rates in different regions of the tumor at different times. This might explain why $q$ was not correlated significantly with the initial drug distribution, whereas $Q$ was. Conversely, the drug partition coefficient, $R$, was correlated significantly with $q$ but not with $Q$, whereas the metabolism rate, $k$, was not correlated with either $q$ or $Q$. Therefore, although the early distribution is flow limited, and the accumulation is perfusion dependent, the metabolism or conversion seems to be independent of both. No significant intratumor conversion of dFdC into its inactive metabolite, dFdU, was encountered in the outflow perfusate. This reaction is catalyzed by cytidine deaminase, an enzyme that is active in liver, kidney, and gut (36) and in human WBC and plasma (19, 24, 26). On this basis, we conclude that 54A and 54B tumors do not exhibit deaminase activity during acellular perfusion. In singular cases in which contamination with host blood was present, a significant conversion into dFdU occurred. The alternative metabolic pathway for parent dFdC is the intracellular phosphorylation into dFdCTP (the active metabolite) by deoxycytidine kinase. In mononuclear blood cells, the intracellular accumulation of dFdCTP is saturable at infusion levels of dFdC of 20 μM, and the elimination of dFdCTP from cells is slow, with a several-hours-long $β$ half-life (25, 26). Grunewald et al. (25) reported a cellular accumulation rate of dFdCTP in the range of 1.8–5.8 μM min$^{-1}$ during infusion with dFdC. These levels were obtained in viable circulating cells. The $k$ values obtained in the present study correspond to a 3-fold lower conversion rate of 0.4–2.0 μM min$^{-1}$. This lower rate was obtained in solid tumors that also comprise significant extracellular compartments and in which a pronounced spatial heterogeneity of perfusion is present (22). For these reasons, one would expect the conversion per volume to be lower in solid tumors than in pure cell suspensions. The deoxycytidine kinase activity in 54A and 54B cells in vitro is not known at present, and the fraction of the total tumor volume that is capable of correlation analysis, which identified flow as the primary determinant of the initial drug uptake. More indirectly, the superiority of the monoexponential modeling of the concentration-time curve also suggests a flow-limited uptake of a diffusible agent in the tumors (32). Thus, our results rule out a significant role of protein binding or membrane binding of the drug as an obstacle for the entry of dFdC into the SCLC tumors. If more than one pharmacokinetic compartment is involved, the current data cannot discriminate it. Previously, we have shown that the tumor uptake of fluorine following an i.p. injection of dFdC is greater in 54B xenografts than in 54A xenografts, using in vivo $^{19}$F magnetic resonance spectroscopy (20), and more recently, using spin helical computed tomography, we found that 54B tumors had slightly larger ($P = 0.06$) rate constants than 54A tumors for the tissue uptake of Hypaque 76, a MW 614 contrast agent (22). This difference was present both in conventional s.c. xenografts.

**Fig. 4** $O_2$ consumption as a function of $q$ in SCLC 54A and 54B tumors. The curves are fitted to a saturable function: $y(q) = aq/(A + q)$, for the estimation of the saturation level. Top, 54A tumors, curve fit; $R^2 = 0.953; a = 170.93 \pm 51.63; A = 2120.77 \pm 805.17$; bottom, 54B tumors, curve fit; $R^2 = 0.814; a = 63.93 \pm 12.86; A = 608.88 \pm 219.33$. 

![Graph](https://example.com/graph.png)
metabolizing dFdC at a given time point also remains unknown. With these reservations, k represents most likely the cellular conversion into dFdCTP. Thus, we conclude that the information derived from our present perfusion experiments is consistent with current knowledge about the pharmacokinetics of dFdC.

In most normal tissues, the pressure-flow relationship is regulated either by neurogenic vasomotor tone or by autoregulation of the vascular bed. When autoregulation is active, the Δp-Q curves are sigmoid with a constant flow, determined by the metabolic demands. Increasing vasomotor tone will shift the curve to the right and will produce a decreasing slope and an increased P0 (29). The linearity of our Δp-Q curves is indicative that neither of those regulatory mechanisms is operative in the vasculature of the two SCLC tumor lines; however, because the actual pressure range over the tumors, following correction for the tubing resistance, is quite narrow (Fig. 3, left curve), the existence of some regulation cannot be excluded based on the current data.

In various transplanted rodent and human tumors, an interstitial fluid loss from the tumor periphery has been demonstrated (35, 37). The reported values are usually in the range of 6–14% of the plasma entering the tumor that oozes out into the surrounding normal tissue. Similar estimates are reported only rarely in perfused rat tumor studies, except for the one by Ohkouchi et al. (15), in which the mean fluid loss was more than 40%. In that particular case, the concentration of the drug in the samples was still likely to be representative of the tumor outflow concentration, because the fluid loss was reported to be mainly due to venous leakage, i.e., the loss occurred after passage through the tumor exchange vessels (15). In the current series of experiments, the tumors were kept wrapped in their paraffin bags during perfusion, and the criteria applied for inclusion in the analysis were directed strictly and specifically at avoiding excess leakiness, as outlined above.

The O2 consumption rates were comparable with those reported for a variety of transplanted rodent tumors and xenografted human tumors. The finding of a possible saturation of the capacity for O2 use (Fig. 4) indicates that, at least in the 54B tumors, the oxidative metabolism is not only flow limited. In theory, an increased fluid loss at higher flow levels might also contribute to the plateau formation, but fluid loss would lead to underestimates of both the perfusion rate and the O2 consumption. Because the fluid loss did not seem to be different in 54A and 54B tumors, any significant contribution from this potential bias to the observed differences in estimated maximal V02 is unlikely. The plateaus can be verified experimentally only with specific O2-carrying agents in the perfusate (e.g., RBC, modified hemoglobins, and perfluorocarbons).

Because the O2 consumption was linearly dependent on the perfusion rate, within the current range, processes other than respiration must have contributed to the increase in pCO2 during passage through a tumor, because the ΔpCO2 was correlated inversely with the flow. The ΔpCO2, estimated on the basis of differential pCO2 readings, is at the least composed of the respiratory CO2 and the amount of CO2 released by the left shift of the equilibrium of the bicarbonate buffer, induced by an increase in H+:

$$\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \quad (G)$$

The lactate production was not correlated significantly with the ΔpH or to the ΔpCO2. This suggests that other sources of H+ must have contributed to the arteriovenous pH difference. This goes along with recent observations in glycolysis-deficient (variant) tumor cells that formed tumors with a degree of acidity comparable to the nondeficient parental cells, indicating that lactate production is not the only mechanism responsible for the development of an acidic environment within solid tumors (38). Lactate production was significantly higher in 54B tumors than in 54A tumors, whereas no significant difference in glucose consumption was observed. This can be interpreted as the result of a higher glycolytic activity in 54B tumors than in 54A tumors, but this explanation does not account for the ratio of lactate-glucose sufficiently.

The abundant lactate production and concurrent O2 consumption are characteristics of the so-called aerobic fermentation, proposed by Warburg (39) as a possible characteristic of solid tumors. Gullino et al. (40, 41) pointed out that as long as the V02 is proportional to the perfusion rate, the supply situation rather than damaged respiration is the principal limiting factor for the O2 consumption, V02, in tumors. Our data suggest a saturation of the O2 consumption at relatively high flow levels in both tumor lines (Fig. 4). These levels correspond to O2 consumption rates of several normal tissues and to rates reported in rodent tumors (40, 42) and human xenografts in nude rats (4), although they are slightly higher than those of human breast tumors (3). Previous studies have used blood or other RBC-containing perfusion media, in which the price for a greater O2-carrying capacity is a lower perfusion rate due to much more complex interactions of rheological parameters (27, 35).

The observation that 54A tumors seem to have a higher capacity for O2 consumption, along with the greater lactate production of 54B tumors, points to a greater glycolytic activity of 54B tumors relative to an oxidative energy metabolism. Our previous finding of a significantly higher steady state ATP content in 54A tumors than in 54B tumors, both when grown as solid tumors and in cell cultures, could be a reflection of such a difference in the balance between glycolysis and respiration. However, the fact that the lactate production in several cases exceeded twice the glucose consumption implies that sources other than the glucose content of the perfusate were metabolized into lactate. Either the mobilization of tumor glycogen, which was found to be present in 54A and 54B tumors, or a catabolic state leading to metabolism of endogenous amino acids might have occurred. Further studies, using specific O2-carrying perfusion media in combination with magnetic resonance spectroscopy, will elucidate these aspects. Using an earlier rat tumor preparation, Eskey et al. (13) found that the RC3230AC mammary carcinoma maintained the tumor nucleotide triphosphate:P ratio following removal of O2 from the perfusate, whereas glucose deprivation led to an immediate drop in this ratio, which returned to normal on reintroduction of glucose in the perfusate. The present SCLC tumor lines produced lactate in excess of what could be accounted for by the glucose consumption. Detailed analysis of metabolic fluxes, using 13C-enriched substrates and 13C magnetic resonance spectroscopy, would
elucidate the metabolic pathways underlying this apparent overproduction of lactate.

We have found that the initial intratumor distribution of dFdC is flow dependent, and the accumulation of the drug is dependent on the perfusion rate, whereas the intracellular activation seems to be independent of physiological parameters. We suggest that attempts to improve the local drug uptake in tumors should be aimed at making the tumor flow distribution more uniform. The present experimental system is ideally suited to preclinical evaluation of such attempts and, more generally, to any approach directed at modifying the local drug delivery and/or metabolism in solid tumors of human origin.

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Intratumor pharmacokinetics, flow resistance, and metabolism during gemcitabine infusion in ex vivo perfused human small cell lung cancer.


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