High-Resolution Magnetic Resonance Imaging of Disparities in the Transcapillary Transfer Rates in Orthotopically Inoculated Invasive Breast Tumors

Maya Dadiani, Raanan Margalit, Noa Sela, and Hadassa Degani
Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel

ABSTRACT

In vivo mapping of the transcapillary fluxes in tumors can help predict the efficacy of delivery of blood-borne anticancer drugs. These fluxes are primarily affected by the vascular permeability and the pressure gradients across the blood vessels’ walls. We describe herein high-resolution dynamic contrast-enhanced magnetic resonance imaging of the influx and outflux transcapillary transfer rates in vivo in invasive MDA-MB-231 tumors orthotopically inoculated in severe combined immunodeficient (SCID) mice. The tumors were noted for rapid growth, impaired drainage of fluid, and subsequent formation of cysts. Consequently, the time evolution of the contrast enhancement, induced by i.v. injection of Gadolinium diethylene-triamine-penta-acetate, exhibited two distinct patterns: transcapillary transfer in the cellular regions and simple diffusion in the cyst fluid. Both processes were analyzed at pixel resolution applying to each a physiological model and a corresponding algorithm. In the cellular region, the influx and outflux transcapillary transfer rates decreased during tumor growth; however, an increased disparity between the transfer constants was observed, with the outflux rate exceeding the influx rate. This quantitative spatial and temporal mapping of this disparity can provide a means to assess the physiological barriers to tracer delivery. It is hypothesized that both the increased disparity in transcapillary transfer rates and impaired fluid drainage in these tumors could arise from the development of interstitial hypertension.

INTRODUCTION

Tumor growth and development relies on the formation of a capillary network that serves to supply nutrients and oxygen to the cancerous tissue, as well as to provide escape routes by which invasive cells can metastasize to distant sites (1). Tumor vasculature, however, is not an ideal network; its rapid and irregular formation results in a disorganized and tortuous architecture, as well as fragile and leaky vessels that lead to both impaired oxygen and nutrient perfusion and impaired drainage of fluids (2). As a result, tumor tissue often develops severe abnormalities such as hypoxic and acidic loci and large necrotic areas, as well as the formation of cysts or edemas and regions of elevated interstitial fluid pressure (IFP) (3). The latter feature, in particular, is a major obstacle to the successful delivery of chemotherapeutic agents to the tumors. Comprehensive studies by Jain et al. (3, 4) have demonstrated that experimental tumors in animal models and several human tumors develop elevated levels of IFP during growth.

Overall, the aforementioned vascular and tissue abnormalities lead to the inadequate delivery of diagnostic and therapeutic agents to solid tumors (3). Therefore, quantifying these abnormalities is of major importance for predicting the efficacy of chemotherapeutic drug delivery, in particular, in breast cancer because neoadjuvant, preoperative treatment is increasingly being used (5, 6). Quantification of transport-related parameters in vivo during tumor growth and progression, as well as in response to therapy requires the use of high-resolution noninvasive imaging methods. Although in vivo microscopy provides the desired spatial resolution required to monitor events at the cellular level, this method is depth limited. Dynamic contrast-enhanced magnetic resonance imaging (MRI) effectively provides means to measure the physiological parameters of perfusion in a noninvasive manner (Refs. 7–11 and references cited therein). This method has been extensively used to diagnose breast and other tumors in preclinical (12, 13) and clinical (9, 14) studies. Dynamic acquisition of images following injection of a contrast agent enables the tracking of tracer uptake and clearance over time, as well as providing information about tissue perfusion. In a recent study, the uptake kinetics of Gadolinium-diethylene-triamino-pentaacetic acid (Gd-DTPA) as measured by dynamic contrast-enhanced MRI were shown to correlate with the delivery of the anticancer agent phenyl acetate (15).

The uptake of diffusible tracers such as Gd-DTPA has been extensively studied, using model-based equations to describe physiological parameters (16–18). Here, we used the generalized scheme proposed by Tofts (18) that uses a compartmental model to describe the transfer of the contrast agent from the intravascular to the interstitial space and back, thus yielding the transcapillary influx and outflux transfer constants, respectively. In most previous studies, it was assumed that the transfer constant is the same in both directions; however, disparate influx and outflux transfer constants more accurately describes the dispersal of the contrast agent into and out of the interstitium, particularly when there are differences in diffusion or pressure on either side of the capillary walls (18). This disparity is more likely to be present in tumors characterized by high interstitial pressure.

In this article, we present data from MRI studies of Gd-DTPA perfusion and diffusion in MDA-MB-231 breast tumors orthotopically inoculated in severe combined immunodeficient (SCID) mice. These tumors exhibited rapid growth accompanied with impaired drainage and formation of cysts and revealed invasive features which includes the formation of metastases. Analyses of dynamic contrast-enhanced MR images of the tumors revealed two distinct patterns of the contrast agent uptake: transcapillary exchange in the cellular regions and diffusion in the cyst. Each process was, therefore, analyzed according to a related model-based equation to yield the appropriate physiological parameters. Monitoring changes in these parameters enabled us to follow alterations in tumor perfusion during growth.

MATERIALS AND METHODS

Mice and Tumors. Human MDA-MB-231 breast cancer cells were obtained from the American Type Culture Collection (Rockville, MD) and were cultured as recommended by the supplier. Cells (2 × 10⁶ to 1 × 10⁷) suspended in 0.2–0.5 ml PBS, were inoculated into the mammary fat pad of female CB-17 SCID mice, 7–10 weeks old.

During the experiments, mice were anesthetized by inhalation of 1% Isoflurane (Medeva Pharmaceuticals PA, Inc., Bethlehem, PA) in an O₂:N₂O (3:7) mixture, applied through a nose cone. After euthanasia, tumors were surgically removed, fixed with 4% formaldehyde, and sectioned in a plane parallel to that of the MRI. Tumors were then embedded in paraffin, sectioned to obtain 4-μm
slices, and stained with H&E. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Weizmann Institute of Science.

**Contrast Agent Pharmacokinetics.** Gd-DTPA (Scherer, Berlin, Germany) was injected as a bolus into the tail vein of the mice, at a dose of 0.5 mmol/kg body weight. Changes in the blood concentration of the contrast agent after injection can be monitored in vivo (19) or ex vivo (20). We used the latter method in a separate group of SCID mice by drawing blood samples from the anesthetized mice by retro-orbital sinus puncture. Measurements of the T2-relaxation times of the plasma samples and calculations of the pharmacokinetic parameters were performed as described previously (12). Table 1 summarizes the values of the pharmacokinetic parameters obtained for SCID mice.

**MRI.** All MR images were acquired with a 4.7-T Biospec spectrometer (Bruker Biospin, Rheinstetten, Germany). For the in vivo studies, mice were placed inside a 1H radiofrequency resonator with an inner diameter of 7.5 cm. Before the imaging protocol, a catheter was inserted into the tail vein of the mice for injection of the contrast agent.

T1-relaxation times of MDA-MB-231 tumors were measured in vivo by applying a spin-echo imaging sequence with a constant echo time (TE) of 15 ms and a series of variable repetition time (TR) values in a range of 150-5000 ms. T2-relaxation times were measured using a constant TR of 3200 ms and TEs ranging from 18-146 ms. Average proton relaxation times in cellular regions of the tumors are summarized in Table 1.

The protocol of each dynamic study included an initial multislice rapid acquisition with relaxation enhancement (RARE) T2-weighted sequence, with settings: TE/TR of 4.3/18.3 ms; one acquisition; flip angle 30°; a typical enhancement ranges (see Supplementary Material). It was found that possible deviations due to the usage of the fast exchange approximation were negligible.

The time course of signal enhancement during the 20 min after bolus injection was calculated at pixel resolution, using a nonlinear Levenberg-Marquardt least-square fitting algorithm (12). The quality of the fit was assessed by calculating a correlation coefficient value R² for each pixel (12). The output provided parametric maps of the influx transfer constant $k_{in}$ and the efflux rate constant $k_{out}$ using a lower limit for the interstitial volume fraction $v_i$ of 0.2 (30).

**Diffusion Studies.** The diffusion coefficient of Gd-DTPA in the cysts, which formed in the tumors, was estimated by adapting the “null point” method and tracking the displacement of a specified concentration of Gd-DTPA as it diffused through the cyst fluid (31). In short, the diffusion of Gd-DTPA in a homogenous medium is given by the following solution to Fick’s diffusion equation (32) using predetermined initial conditions and boundaries (31):

$$x = 2(D^{0.5})[\text{erf}^{-1}(1 - C_{null}/C_0)]^{0.5}$$

for $t = 0; C_0 > 0$ at $x > 0$, and for $t > 0; C = C_0$ at $x = 0 = C = x = 0$, where $D$ is the diffusion coefficient, $x$ is the distance from the start of the tracked route that at time $t$ reached a constant value of concentration $C_{null}$. $C_0$ is an initial steady concentration of Gd-DTPA at $x = 0$, and erf$^{-1}$ designates the inverse error function.

$C_0$ was estimated as the average concentration of Gd-DTPA at the boundaries of the cyst 5 min after injection. Different routes taken by Gd-DTPA from the edge of the cyst toward its center were tracked, and the time to reach 20% signal enhancement (corresponding to $C_{null} = 0.03$ mmol Gd-DTPA) in each pixel along these routes was determined. The distance $x$ of each pixel from the edge of the cyst versus the square root of this time yielded linear plots. For each tumor, 6–10 plots were created, one for each extracted route. The diffusion coefficient was then determined from the corresponding slope, using equation B. The mean value of the diffusion coefficient was calculated by averaging values obtained for tumors of similar age.

**RESULTS**

**MDA-MB-231 Tumor Growth and Invasiveness.** Initially, we aimed to characterize the growth patterns and histopathological features of MDA-MB-231 tumors. Solid, palpable tumors developed within a week after inoculation of cells into the mammary glands of SCID mice. The presence and size of the tumors were monitored by T2-weighted MRI images. Each tumor was monitored twice, first within a week after inoculation of cells into the mammary glands of SCID mice, severe combined immunodeficiency (n = 12).

**Table 1 Parameters used in analyzing the dynamic Gd-DTPA-enhanced MR images in MDA-MB-231 tumors inoculated in SCID mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>mmol/kg wt</td>
<td>0.57</td>
</tr>
<tr>
<td>Pharmacokinetic parameters of Gd-DTPA in plasma of SCID mice (equation A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$a_1$ [kg/liter]</td>
<td>Amplitude of fast decay</td>
<td>7.91</td>
</tr>
<tr>
<td>$a_2$ [kg/liter]</td>
<td>Amplitude of slow decay</td>
<td>6.96</td>
</tr>
<tr>
<td>$m_1$ [min$^{-1}$]</td>
<td>Fast decay time constant</td>
<td>1.68</td>
</tr>
<tr>
<td>$m_2$ [min$^{-1}$]</td>
<td>Slow decay time constant</td>
<td>0.06</td>
</tr>
<tr>
<td>Nuclear relaxation times of MDA-MB-231 tumor tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_1$ [s$^{-1}$]</td>
<td>T1 Relaxivity</td>
<td>4.3</td>
</tr>
<tr>
<td>$R_2$ [s$^{-1}$]</td>
<td>T2 Relaxivity</td>
<td>4.9</td>
</tr>
<tr>
<td>$T_{1p}$ [s]</td>
<td>Tumor $T_1$ relaxation time</td>
<td>1.7</td>
</tr>
<tr>
<td>$T_{2p}$ [s]</td>
<td>Tumor $T_2$ relaxation time</td>
<td>0.07</td>
</tr>
</tbody>
</table>

$^a$ Gd-DTPA, Gadolinium-diethylene-triamino-pentaacetic acid; MR, magnetic resonance; SCID, severe combined immunodeficiency.

$^b$ Parameters were determined as described above. Relaxivity rates were determined previously (12).

$^c$ Pharmacokinetic curve fitting $R^2 = 0.93$ (n = 18).

$^d$ Relaxation times $T_1$ and $T_2$ curve fitting with $R^2$ 0.99 and 0.98, respectively.
and limb bones, ~45 days after inoculation of 10^7 tumor cells into the mammary fat pad of the mice.

**Dynamic MRI of Transcapillary Transport.** The transcapillary transfer rates of the contrast agent Gd-DTPA were measured in the tumors over the course of their growth by dynamic contrast-enhanced MRI. Fig. 2 demonstrates the time evolution of Gd-DTPA uptake as seen by signal enhancement post contrast-agent injection in MR images of a fast-growing MDA-MB-231 breast tumor, 8 days after inoculation. Temporal differences in uptake and clearance of the contrast agent were observed throughout the entire tumor and were particularly distinct between the cyst and the viable cellular regions (Fig. 2, C and D). The cyst region is clearly delineated in the corresponding T2-weighted image (Fig. 2A). The viable areas demonstrated rapid enhancement by the contrast agent followed by a slow decay, whereas the enhancement of the contrast agent seen within the cyst was delayed and far more gradual.

The dynamic MRI data were analyzed using a multicompartment perfusion model (18) with a different influx transfer constant, k_in, and outflux transfer constant, k_out (detailed in “Materials and Methods”). The model yielded parametric maps of these constants, showing extensive good fitting with a correlation coefficient R^2 ≥ 0.5 in the viable regions and failed to fit at cyst or nonperfused regions. A typical example of the change in the k_in and k_out transfer rate constants during tumor growth is illustrated by color-coded parametric images of these constants (Fig. 3). A clear reduction in k_in and less pronounced in k_out was observed during growth. The k_out/k_in ratio maps presented the extent of disparity in the fluxes.

Histograms of both transfer constants values (for pixels with R^2 ≥ 0.5) in the tumor demonstrated a heterogeneous, skewed distribution; hence, statistical analysis was based on calculating the 25th, median, and 75th percentiles of each parameter (Table 2). During tumor growth, a significant decrease (P ≤ 0.05) in the values of k_in and k_out at all percentiles was observed (Table 2 and Fig. 4). In parallel, however, the ratio k_out/k_in that reflected the disparity in the transcapillary fluxes of the contrast agent increased significantly with tumor growth (P ≤ 0.05; Table 2 and Fig. 4). This disparity was also revealed by the pixel-by-pixel correlation between k_in and k_out at the two stages of growth of the same tumor, as shown in Fig. 5. The heterogeneity of both constants was high in the early stages of tumor growth. However, as the tumors grew, the disparity in the constants, with k_out > k_in, became much more prominent, as demonstrated by a steeper slope of the regression trend.
A similar disparity was also found in slow growing, MDA-MB-231 tumors (inoculated with $2 \times 10^5$ tumor cells) that reached a smaller size of $0.15 \pm 0.02$ cm$^3$ in 25 days (Fig. 6). In maps of the transcapillary transfer rates from the entire axial slice of the body (Fig. 6, bottom panel), it can be noticed that aside from the tumor viable tissue, most of the pixels did not enhance and hence failed to fit the physiological model. In these tumors, the cysts were small, and the viable area was much broader, enabling observation of detailed spatial distribution of the transcapillary transfer constants. The influx transcapillary transfer constant varied throughout the tumor randomly, whereas the outflux transfer constant showed a distinctive spatial distribution (Fig. 6). This pattern is also shown in the $k_{out}/k_{in}$ that was low at the rim, increased toward the interior areas, but declined again at the boundaries of the cyst.

**MRI of Gd-DTPA Diffusion in the Cysts.** The time evolution of the contrast-agent distribution in the cyst enabled us to measure, in vivo, the diffusion coefficient of Gd-DTPA in an aqueous environment. Parametric maps of the time needed to reach 20% enhancement in the cyst, which corresponded to a concentration of 0.03 mM Gd-DTPA, were constructed as illustrated in Fig. 7B. We measured the time in which 0.03 mM Gd-DTPA reached a distance $x$ from the edge of the cyst in routes selected according to the enhancement maps (Fig. 7B). Plots of the displacement $x$ as a function of the square root of time, $\sqrt{t}$, yielded a linear increase that was in agreement with Fick’s Second Law of Diffusion (32). Fig. 7C shows the diffusivity plots averaged for the five tumors in the two stages of growth. The two resulting slopes were similar, indicating comparable diffusion coefficients in the cyst fluid at both stages of growth (Table 3); however, lower $C_0$ in the large tumors led to a longer delay time.

**DISCUSSION**

In this study, we endeavored to quantify the physiological parameters that describe the transcapillary transfer rates during the growth and development of the invasive MDA-MB-231 breast cancer tumors orthotopically inoculated in SCID mice. Specifically, we demonstrated the capability of characterizing the spatial distribution and temporal changes in transcapillary transfer rates of the clinically used Gd-DTPA contrast agent during tumor growth. Our results demonstrated an overall decrease in the transcapillary fluxes as the tumors developed but a concomitant increase in the disparity between the inward and outward fluxes. We also monitored the time-dependent distribution of Gd-DTPA in the fluid-filled cystic cavities of these tumors, thereby determining Gd-DTPA diffusion coefficient in vivo.

In general, the transcapillary exchange of a tracer molecule between the intravascular and the extracellular spaces occurs by diffusion and convection (3). To separate the effects of these mechanisms and to
quantify the parameters underlying each, it is necessary to track, over time, the changes in the tracer concentration gradient, as well as in the pressure gradient across the capillary walls (33). Alternatively, tracking the global changes in contrast agent distribution, discriminating inward and outward fluxes, enabled us to monitor changes in the transcapillary delivery of the contrast agent during tumor growth, as well as to discover the disparity of the transcapillary exchange.

The analysis of the data using Tofts’ model (18) directly yielded the influx transfer constant of the contrast agent $k_{in}$, as well as the efflux rate, $k_{out}$, which is the quotient of the outflux transfer constant, $k_{out}$, and the extravascular extracellular volume fraction, $v_e$ ($k_{ep} = k_{out}/v_e$). In most previous studies of dynamic contrast-enhanced MRI, it was generally assumed that the influx and outflux transfer constants were similar ($k_{in} \approx k_{out}$); consequently, the two parameters $k_{in}$ and $v_e$ could be readily obtained by fitting the data to the proper equations (12, 17). Analysis of our data using this assumption yielded a large fraction of $v_e < 0.1$. These excessively low $v_e$ values indicated that the transcapillary rate constants might be different, $k_{in} \neq k_{out}$. Microscopic examination and analysis of the histological slices of the tumors revealed high cell density in the viable regions in the MDA-MB-231 tumors; accordingly, we estimated a $v_e$ of $\approx 0.2$, which is the lower limit for this parameter in cancer tissues (30). This value was used in our analysis for estimation of the lower limit for $k_{out}$ from the obtained $k_{ep}$ maps. Our dynamic contrast-enhanced study, therefore, provided absolute transfer constants for the influx transfer constant, lower limits for the outflux transfer constant, and the degree of disparity among the fluxes. Clearly, to obtain accurate values for $k_{out}$ and $v_e$, it is necessary to use some other method to independently estimate $v_e$ at the same resolution as that of the dynamic data.

**Fig. 5.** Correlation between the influx and outflux transcapillary transfer constants. A typical scatterplot of $k_{in}$ versus $k_{out}$ values in all best fit pixels ($R^2 > 0.5$) in a tumor 11 (A) and 22 (B) days after inoculation of MDA-MB-231 cells.

**Fig. 6.** Parametric images of the transcapillary transfer constants in a slow-growing MDA-MB-231 tumor inoculated in severe immunodeficient mouse ($R^2 > 0.5$). The top panel shows only the tumor region, and the bottom panel shows the entire axial slice of the body. The tumor was imaged 25 days after inoculation of $2 \times 10^6$ tumor cells. The parametric images of $k_{in}$ and $k_{out}$ revealed variation in their spatial distribution. Note the spatial distribution of $k_{out}$ relative to $k_{in}$.

**Fig. 7.** Diffusion of Gadolinium-diethylene-triamino-pentaacetic acid within the cyst fluid. A T2-weighted magnetic resonance image of a central slice of a tumor, 11 days after inoculation of $10^7$ tumor cells (A), and map of the time taken to reach 20% enhancement from the edge of the cyst (B). Arrows indicate representative tracks for diffusion selected according to the map for calculating the diffusivity. C, diffusion curves of Gadolinium-diethylene-triamino-pentaacetic acid in the cyst fluid of the fast growing tumors 8–11 days (●) and 18–22 days (▲) after inoculation. The error bars are the SD of the averaged plots from five tumors.
The significant reduction that we observed in the transvascular transfer rates in the course of tumor growth can be explained by decreased vascular permeability. Simultaneous measurements for both flow- and permeability-limited tracer revealed that the perfusion of Gd-DTPA is mainly permeability limited (34). Newly formed microcapillaries in tumors are highly permeable due to primary organization, but remodeling and thickening of the vessels' walls leads to less permeable vessels (1). This progressive process suggests that the duration of tumor growth, rather than tumor size per se, determines the vascular perfusion parameters. The reduction in both the influx and outflux transfer rates as the tumor develops could, therefore, reflect decreased capillary permeability because of vessel maturation.

Interestingly, the decrease in the transfer rates of the contrast agent was not identical for both directions. Rather, the decrease in the influx transfer rate was higher than that of the outflux rate. Consequently, the disparity in the transfer rates increased with tumor growth. A similar disparity was observed in the slow-growing tumors, although they reached smaller size. This suggests that the increase in the disparity is attributed to physiological processes that occurs during tumor development rather than tumor size.

We hypothesize that the increased disparity in transfer rates as the tumor grows is because of increased interstitial hypertension as high interstitial pressure will force fluid to reenter the blood vessels thus increasing outflux to influx ratio. It was previously shown that the difference in hydrostatic pressure across the capillary walls is the main mechanism underlying interstitial hypertension (35). Moreover, IFP has been shown to increase with tumor size and more specifically to be related to the number of days after tumor implantation (35–37). Additionally, the spatial distribution of the disparity between the constants shown in Fig. 6 is in accordance with measurements of IFP, showing elevated levels throughout the tumor that drops in the tumor periphery (38).

We speculate that staging of the tumors as to their disparity between outflux to influx transvascular transfer may correlate with their net physiological barrier to transcapillary transfer and hence drug delivery. Additional investigations, however, are necessary to prove the correlation between the disparity in the transcapillary transfer constants and IFP.

It has been shown that a combination of rapid fluid extravasation and poor drainage of fluid within the tumor leads to the formation of cysts and to the development of IFP (30). Hence, the formation of cysts is also in agreement with the hypothesis that these tumors may develop high interstitial pressure. The dynamics of the contrast agent uptake in the cyst matched a diffusive behavior, thus enabled direct measurement of the diffusion coefficient of Gd-DTPA in vivo, by tracking the displacement of the contrast agent within the cyst fluid. This in vivo measurement yielded a diffusion coefficient of $1 \times 10^{-5}$ cm$^2$/s at body temperature. To the best of our knowledge, the Gd-DTPA diffusion coefficient was previously measured only in polyvinyl chloride hydrogel, which serves as a tissue mimic, yielding a diffusion coefficient of $2.6 \times 10^{-6}$ cm$^2$/s (31). The differing diffusion coefficients seen in the cystic fluid and in the hydrogel are in accord with previous studies in which the interstitial diffusion coefficients of various molecules were shown to be about one-third of those in solution (39). Hence, the estimated time that it took for Gd-DTPA to diffuse within a plane in the interstitium across a distance $L$ of $\sim 200 \mu$m ($\sim$ pixel dimension) was $\sim 32$ s ($t = L^2/4D$). This supports our interpretation of the dynamic data in the cellular regions of the tumors (obtained at a temporal resolution of 37 s), which assumed a fast and nearly even distribution of the contrast agent in the interstitial space of each pixel during the acquisition time.

In summary, our results showed that, by the use of dynamic contrast-enhanced MRI, it is possible to distinguish between the influx and outflux transfer constants and that these constants decrease at different rates as the tumor develops, leading to an increase in disparity between the corresponding transfer constants, with the outflux rate exceeding the influx rate. Extending this approach may help predict the efficacy of delivering molecules with properties similar to those of the contrast agents.

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REFERENCES


TRANSCAPILLARY TRANSFER RATES IN BREAST TUMORS

Table 3 Gadolinium-diethylene-triamino-pentaacetic acid Diffusion in the cyst fluid of MDA-MB-231 tumors in severe immunodeficient mice at the two stages of growth

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>$C_{\text{eff}}$ mm</th>
<th>Slope, cm$^2$/s</th>
<th>Diffusion coefficient, cm$^2$/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-11 days</td>
<td>0.25 $\pm$ 0.05</td>
<td>(6.5 $\pm$ 1.3) $\times 10^{-3}$</td>
<td>(0.9 $\pm$ 0.3) $\times 10^{-3}$</td>
</tr>
<tr>
<td>8-22 days</td>
<td>0.18 $\pm$ 0.08</td>
<td>(6.1 $\pm$ 0.4) $\times 10^{-3}$</td>
<td>(1.2 $\pm$ 0.4) $\times 10^{-3}$</td>
</tr>
</tbody>
</table>

* Days post inoculation of $10^7$ cells, $n = 5$.

The diffusion coefficient was calculated according to equation $B$ from the slope of the traveled distance from the edge of the cyst versus the square root of time to reach a $C_{\text{null}}$ of 0.03 mm (Fig. 6). For each tumor, 6–10 routes tracked from edges of the cyst, in a central slice, were averaged.

with previous studies in which the interstitial diffusion coefficients of various molecules were shown to be about one-third of those in solution (39). Hence, the estimated time that it took for Gd-DTPA to diffuse within a plane in the interstitium across a distance $L$ of $\sim 200 \mu$m (pixel dimension) was $\sim 32$ s ($t = L^2/4D$). This supports our interpretation of the dynamic data in the cellular regions of the tumors (obtained at a temporal resolution of 37 s), which assumed a fast and nearly even distribution of the contrast agent in the interstitial space of each pixel during the acquisition time.

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