Effects of the Vascular Disrupting Agent ZD6126 on Interstitial Fluid Pressure and Cell Survival in Tumors

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Abstract

Interstitial fluid pressure (IFP) is elevated in tumors due to abnormal vasculature, lack of lymphatic drainage, and alterations in the tumor interstitium. ZD6126 is a tubulin-binding agent that selectively disrupts tumor vasculature resulting in tumor necrosis. This study examined the effect of ZD6126 on tumor IFP and the response of tumors with different IFP levels to ZD6126. Pretreatment IFP was measured using the wick-in-needle method in tumors (murine KHT-C and human CaSkii) growing i.m. in the hind legs of mice. Mice were treated i.p. with a single dose of ZD6126 (100 or 200 mg/kg) and posttreatment IFP measurements were made. Blood flow imaging was conducted using Doppler optical coherence tomography, whereas oxygen partial pressure was measured using a fiber optic probe. Clonogenic assays were done to determine tumor cell survival. In KHT-C tumors, IFP dropped significantly at 1 hour posttreatment, returned to pretreatment values at 3 hours, and then declined to ~25% of the pretreatment values by 72 hours. In CaSkii tumors, the IFP decreased progressively, beginning at 1 hour, to ~30% of pretreatment values by 72 hours. Clonogenic cell survival data indicated that ZD6126 was less effective in tumors with high IFP values (>25 mm Hg). Vascular disrupting agents, such as ZD6126, can affect IFP levels and initial IFP levels may predict tumor response to these agents. The higher cell survival in high IFP tumors may reflect greater microregional blood flow limitations in these tumors and reduced access of the drug to the target endothelial cells. (Cancer Res 2006; 66(4): 2074-80)

Introduction

Tumors are characterized by irregular, hyperpermeable vessels generated by tumor angiogenesis, a lack of functioning lymphatics, and an abnormal extracellular matrix (1, 2). Excess fluid leaks from the vasculature into the interstitium, where it accumulates and distends the elastic extracellular matrix, elevating interstitial fluid pressure (IFP). An equilibrium is established where the interstitial pressure approximates the capillary pressure, resulting in reduced transvascular pressure gradients (relative to normal tissue) and reduced fluid movement through the tumor interstitium (3–5). An additional factor affecting IFP levels may be the proliferation of tumor cells in a confined space. Several experimental studies using animal models (6–12) have shown improved drug uptake following reduction of tumor IFP. Although the effect of high tumor IFP on drug uptake in patients has not been shown conclusively, there is strong evidence that high IFP confers a poor prognosis in patients with cervix cancer treated with radiotherapy (13). Furthermore, as elevated tumor IFP has been measured in a variety of human tumors, including metastatic melanoma, breast, head and neck, colorectal, and cervical carcinoma, it represents a critical therapeutic problem that needs to be addressed (13–18).

Compounds that have been shown in laboratory experiments to reduce IFP include inhibitors or antagonists of proteins that are involved in regulating IFP, such as vascular endothelial growth factor (VEGF; ref. 6), platelet-derived growth factor (7, 8), and transforming growth factor-β (19). An alternative approach is to target the existing tumor vasculature, which plays a key role in the pathogenesis of elevated IFP. The tumor vasculature is a known requirement for tumor survival and proliferation and thus has long been designated as a potential therapeutic target. Antivascular strategies can be divided into two broad categories: antiangiogenic agents that target the developing neovasculature and vascular disrupting agents that attack the existing vasculature.

One example of a vascular disrupting agent is ZD6126, a prodrug that is rapidly converted to its active form, N-acetylcyclochinol (ZD6126 phenol), by serum phosphatases (20). ZD6126 phenol binds to and destabilizes tubulin, causing morphologic changes in endothelial cells (20–22). This results in increased microvascular permeability and probably also occlusion of the precapillary vessels that feed the tumor, leading to a cascade of reduced blood flow, vascular congestion, thrombus formation, metabolic starvation, and tumor cell necrosis (20, 22–25). This process is very rapid and there is evidence of vascular congestion in experimental tumor models by 1 hour posttreatment followed by substantial central necrosis within 24 hours (26). Despite the rapid induction of central necrosis in these models, a rim of viable malignant cells persists at the tumor-normal tissue interface (23, 26–29). Tumor selectivity is apparently achieved through a greater effect on the immature, proliferating endothelial cells that comprise the neovascularization of tumors, compared with the quiescent endothelial cells of the normal vasculature (21–23, 30, 31). In addition, the rapid clearance of the drug from the circulation with a half-life of 1 to 2 hours is thought to be pivotal in reducing toxicity in normal cells.

To date, the effects of vascular disrupting agents on IFP have not been extensively studied and the reported results have been contradictory. Eikesdal et al. (25) found no change in IFP 3 hours after treatment of BT4An rat gliomas with combretastatin A-4, another vascular disrupting drug. In contrast, Hori et al. (24) showed a marked reduction in IFP 30 minutes after administration of the combretastatin derivative AC7700 to L5Y80 sarcomas, with partial recovery of IFP by 6 hours. In general, the effects of vascular disrupting drugs on vessels and the interstitium should
lead to changes in IFP. These agents increase capillary permeability (25), which would tend to increase IFP, whereas the development of central necrosis would increase interstitial hydraulic conductivity and lower IFP (32). However, the magnitude of the IFP in individual tumors probably also depends on the relative values of the precapillary and capillary blood flow resistance (33). Early distortion in the shape of endothelial cells with vessel occlusion, and intravascular coagulation at later time points, would both be expected to decrease blood flow. However, the former should be associated with a decline in IFP because of higher precapillary geometric blood flow resistance, whereas the latter should cause an increase in IFP because of increased capillary viscous resistance (33).

The aim of this study was to investigate the effect of ZD6126 on tumor IFP and the effect of tumor IFP on treatment response. Studies were carried out using a murine fibrosarcoma (KHT-C) and a human cervix cancer xenograft model (CaSki). Both tumors showed a time-dependent reduction in IFP following treatment with ZD6126, and in both cases malignant cells in tumors with high initial IFP were less sensitive to the drug.

Materials and Methods

Mice and tumor cell lines. Experiments were done using the previously described KHT-C murine fibrosarcoma cell line (34) and the CaSki human cervical carcinoma cell line obtained from the American Type Culture Collection (Manassas, VA). Both cell lines were maintained on an alternating in vitro/in vivo growth cycle. In vitro cells were maintained as monolayers in plastic tissue culture flasks using α-MEM medium (Life Technologies, Inc., Burlington, Ontario, Canada) supplemented with 10% fetal bovine serum (FBS, Wisent, Quebec, Canada). Cells between their second and fifth in vitro passage were removed from the flasks during exponential growth using 0.05% trypsin for 5 minutes at 37 °C and transplanted into the appropriate mice. KHT-C cells were transplanted into syngeneic 8- to 12-week-old C3H/HeJ male mice (The Jackson Laboratory, Bar Harbor, ME). CaSki cells were transplanted into 8- to 12-week-old male severe combined immunodeficient (SCID) mice obtained from an in-house breeding program. For all of the experiments, except those using Doppler optical coherence tomography (DOCT) to measure blood flow, the tumors were initiated in the left gastrocnemius muscle following injection of $2.5 \times 10^5$ cells in a 50 μL volume of α-MEM media. Tumor growth was monitored by measurement of the external leg diameter. Animals were housed at the Ontario Cancer Institute animal facility and had access to food and water ad libitum. All experiments were done under protocols approved according to the regulations of the Canadian Council on Animal Care.

Vascular disrupting agent ZD6126. ZD6126 was provided by AstraZeneca (Macclesfield, United Kingdom). For administration to the mice, it was dissolved in 10% sodium carbonate and 90% saline (pH 7.4) and injected i.p. at concentrations of 100 or 200 mg/kg in a volume of 0.01 mL/g body weight.

IFP measurements. IFP was measured using a wick-in-needle technique (35). Briefly, a 23-gauge needle with a custom-ground 1 to 2 mm side port was connected through 1 m of PE22 polyethylene tubing (Becton Dickinson, Franklin Lakes, NJ) to a pressure transducer (model P23XL, Viggo-Spectramed, Oxnard, CA) and an electronic data acquisition and recording system (Model MP100, World Precision Instruments, Sarasota, FL). A “wick” in the form of a multifilamentous cotton thread was placed in the distal portion of the needle and the entire system was flushed with a heparin/sulfate/saline solution (1:10). The system was calibrated before and following each series of measurements by varying the position of the needle tip a known distance above or below a reference elevation. Measurements were initiated in unanesthetized animals once the tumors attained a size of 0.7 to 0.9 g (11-12 mm in diameter). Following a pretreatment IFP measurement, a single dose of ZD6126 was administered to the experimental mice. Control mice received an equal volume of saline. Subsequent IFP measurements were made at 1, 3, 24, 48, and 72 hours posttreatment in the same tumors. At each time point, IFP measurements were taken at three to four different locations in the tumor to address spatial heterogeneity and the mean value of these readings was taken to represent the tumor IFP. Both the KHT-C and CaSki tumors showed significant variability in IFP values among individual tumors (range: KHT-C 7-59 mm Hg; CaSki 15-40 mm Hg), which allowed the influence of different IFP values on treatment response to be assessed.

Anesthetic. To conduct blood flow and oxygen measurements, mice were anesthetized using a combination of Innovar-Vet administered i.p. (0.05 mL/mouse of 1:100 solution; M.T.C. Pharmaceuticals, Mississauga, Ontario, Canada) and Ketalar administered i.m. (ketamine hydrochloride 25 mg/kg; Bimeda-MTC Animal Health Inc., Cambridge, Ontario, Canada).

Blood flow measurement with DOCT. Blood flow measurements were acquired noninvasively in anesthetized mice bearing intradermal tumors 5 to 6 mm in diameter using DOCT (36, 37). OCT (38) is analogous to ultrasound imaging; however, it uses near-IR light waves instead of sound waves to form micrometer-scale resolution subsurface tissue images. The imaging depth of OCT is ~2 mm in most mammalian tissues. Its Doppler extension, known as DOCT, is used to detect subsurface motion, attributable to microvascular blood flow in living specimens. DOCT is thus a dual-imaging platform capable of furnishing high-resolution microstructural tissue maps overlain with blood flow information at the microcirculation level. Its noninvasive nature allows continuous monitoring and longitudinal studies and to ability to detect treatment-induced changes in microvascular flow was used in this study. Specifically, before treatment with ZD6126, blood vessels were identified on DOCT and images acquired for 10 minutes to establish the baseline for the Doppler imaging and to assess the effect of anesthetics. Although the imaging continued, the drug was administered i.p. and blood flow was monitored for an additional 30 minutes. A similar procedure without the ZD6126 injection was followed for control animals.

Measurement of oxygen partial pressure. Tumor oxygenation was measured near the center of the tumor using fiber optic probes connected to the OxyLite 4000 system (Oxford Optronix Ltd., Oxford, United Kingdom). The probe contains a luminescent dye at the tip for which the signal half-life is inversely proportional to oxygen concentration. The data-collecting device excites the dye with a light pulse and then measures the signal half-life and converts it to a oxygen partial pressure ($P_O_2$) value. The Oxylite probe was introduced into the tumors of unanesthetized mice using a 20-gauge needle to protect the tip. Measurements of $P_O_2$ were taken continuously for 10 minutes before drug treatment and for 1 hour posttreatment.

Measurement of cell survival. Mice were sacrificed at 24, 48, or 72 hours after treatment with ZD6126 and the tumors were excised for clonogenic survival assay. The assays were carried out in individual tumors as described previously (39). Briefly, excised tumors were mechanically and enzymatically digested to form single cell suspensions. Cell suspensions were counted with a hemocytometer using trypan blue exclusion to ensure plating of viable cells. Appropriate dilutions of the cell suspensions were plated in triplicate on 100 mm plastic tissue culture plates containing 10 mL α-MEM medium supplemented with 10% FBS. Plates were stained 10 to 12 days later using a 1% methylene blue solution in a 50:50 mixture of ethanol/water. Colonies containing ≥50 cells were counted. Survival was calculated as the number of colony-forming cells recovered per treated tumor. All tumors were the same size at the time of treatment (0.7-0.9 g).

Histologic analysis. KHT-C and CaSki tumors in experimental and control mice were excised pretreatment and at 1, 3, 24, 48, and 72 hours posttreatment. The tissues were fixed in neutral buffered formalin. Sections from each tumor were taken at three levels and stained with H&E. The proportion of necrosis and the size of the peripheral viable rim of cells were evaluated qualitatively for each KHT tumor by imaging the H&E-stained slides under a Nikon upright microscope (Nikon OPTIPHOT model) in the Advanced Optical Microscopy Facility at the Ontario Cancer Institute. Images of the slides were acquired using a CoolSnap Pro color camera and Image Pro Plus software.

In addition, the thickness of the viable rim of cells was measured in six KHT tumors 24 hours after ZD6126 administration, and in six KHT tumors...
48 hours after ZD6126. For each tumor, three regions of the viable rim at its maximal thickness were identified and the number of cell nuclei across the rim was counted by an observer blinded to treatment.

**Statistical analysis.** Correlations between two variables were examined by linear regression analysis. Relationships among three or more variables were evaluated using ANOVA followed by the Dunnett’s multiple comparison test. The data sets were verified to comply with conditions of normality and were tested for equal variance using Bartlett’s test for equal variance. $P < 0.05$ was considered significant. The statistical analysis was done using GraphPad Prism statistical software (GraphPad Software Incorporated, San Diego, CA).

**Results**

**ZD6126 treatment reduced tumor IFP in both KHT-C and CaSki tumors.** Tumor-bearing mice received ZD6126 in single doses of either 100 or 200 mg/kg. Pretreatment IFP measurements were taken, followed by posttreatment measurements at 1, 3, 24, 48, and 72 hours to track the temporal effect of the drug on IFP. Figure 1 shows the temporal changes in IFP following administration of ZD6126 with the values normalized to the pretreatment value for each tumor. Figure 1A shows temporal fluctuations in IFP in KHT-C tumors following administration of 100 and 200 mg/kg of ZD6126. Tumors treated with 200 mg/kg of ZD6126 showed a significant (50%, $P < 0.01$) decrease in IFP within 1 hour of drug administration. By 3-hour posttreatment, IFP levels had returned to, or exceeded, pretreatment values, before declining again at 24 hours posttreatment to 60% of the pretreatment value. Statistical analysis showed no significant difference between the IFP values at 1 and 24 hours posttreatment ($P = 0.15$); however, a statistically significant difference was observed between the IFP values at 3 and 24 hours posttreatment ($P < 0.01$). The decrease in IFP continued progressively at 48 and 72 hours resulting in IFP values that were 25% of those seen before treatment. The changes in IFP were similar although of lesser magnitude following a dose of 100 mg/kg.

In CaSki tumors, IFP values declined continuously following ZD6126 treatment (Fig. 1B). As for the KHT-C tumors, IFP levels were reduced within 1 hour of drug treatment, although to a lesser extent. There was no recovery at 3 hours; instead, IFP levels continued to decline gradually over time, reaching ~30% of the initial pretreatment value by 72 hours posttreatment.

Figures 2A and B show the pretreatment versus posttreatment IFP values in individual tumors at 48 hours after treatment with ZD6126 (200 mg/kg). The 48 hours posttreatment IFP decreased by the same proportional amount regardless of the pretreatment value.
ZD6126 treatment reduced blood flow and oxygenation in KHT-C tumors. To confirm results of others that ZD6126 is indeed reducing blood flow, we measured tumor blood flow and tumor oxygenation. DOCT imaging was used to follow regional tumor blood flow following administration of ZD6126 at 200 mg/kg in 15 KHT-C tumors. Preliminary studies showed that the use of anesthetics did not significantly alter tumor IFP or the DOCT results (data not shown), and thus the mice were anesthetized before treatment to prevent movement artifact. Imaging was initiated 10 minutes before drug treatment, followed by continuous imaging of the same region over a 30-minute period posttreatment. Figure 3 shows a representative plot of blood flow over time in a treated tumor. The vertical axis shows a normalized pixel count of pixels exhibiting a flow-induced Doppler shift above the noise floor, a useful metric of flowing blood volume fraction. ZD6126 treatment elicited a rapid decline in blood flow starting at ~7 minutes posttreatment. By 20 minutes, blood flow had ceased in the imaged vessels and remained shutdown over the following 10-minute imaging period. In all 15 tumors studied, the DOCT signal fell to zero over the same time period. The normalized Doppler pixel count did not change significantly over the corresponding ~40-minute DOCT imaging interval in untreated control animals (n = 7, data not shown).

Oxygen measurements were made in 10 KHT-C tumors treated with ZD6126 (200 mg/kg) using an Oxylite probe for 10 minutes before drug administration and for 1 hour afterward. The mice were anesthetized to prevent movement. All of the tumors displayed the same response to ZD6126. Figure 4 shows a representative plot of $p_O2$ measured over time. $p_O2$ was stable until 20 to 25 minutes posttreatment, when it began to decline. This matched the time point at which complete shutdown of blood flow was apparent (Fig. 3). By 30-minute posttreatment, $p_O2$ had reached 0 mm Hg and remained so for the duration of the measurement. In contrast, untreated tumors showed relatively consistent $p_O2$ values over the entire measurement period (n = 8).

ZD6126 treatment induced extensive central necrosis in KHT-C tumors. Previous studies using ZD6126 have shown a characteristic pattern of complete central necrosis with a rim of viable malignant cells at the tumor periphery (22, 30, 31). The blood flow and $p_O2$ data (Figs. 3 and 4) suggest complete vascular shutdown. Consequently, a total of 12 KHT-C tumors were excised following treatment with ZD6126 for histologic analysis by an observer blinded to the IFP values and the findings were similar in all cases. Figure 5 shows histologic sections from representative tumors at different time points before and after treatment with ZD6126 (200 mg/kg). There was no apparent histologic change at 1 hour. However, by 3 hours, there was evidence of vascular congestion and hemorrhage. At 24 hours, there was extensive central tumor necrosis and a persistent rim of malignant cells with a maximum thickness of ~10 cell diameters (Table 1). By 48 hours posttreatment, the maximum viable rim thickness had expanded to ~25 cells in keeping with tumor cell repopulation. This is equivalent to approximately one doubling of the surviving cells assuming a spherical tumor (10 mm diameter). There was no apparent relationship between the pretreatment IFP and the thickness of the rim. Control tumors showed no evidence of excessive necrosis beyond that normal for a tumor of this size and type at any of the time points studied.

Sections of 10 ZD6126-treated CaSki tumors were studied in a similar manner. CaSki tumors develop more necrosis during normal growth than KHT tumors, and no difference in the amount of necrosis were observed at 1 and 3 hours between drug-treated and control tumors. At 24 hours, treated CaSki tumors showed extensive central necrosis and discontinuities in the viable rim. However, by 48 hours, the viable rim was more prominent and there were larger regions of viable-appearing tissue.

Elevated tumor IFP results in enhanced survival following treatment with ZD6126. Tumor cell survival in response to treatment with ZD6126 was assessed using clonogenic survival assays. Tumors were excised 48 hours posttreatment with ZD6126 at doses of 100 and 200 mg/kg. Tumor cell survival was calculated based on the number of colonies formed following plating of a known number of viable cells from the total tumor. Figure 6 shows survival data plotted against pretreatment IFP for both KHT-C (Fig. 6A) and CaSki (Fig. 6B) tumors. The number of clonogenic tumor cells was lower in both treated and untreated CaSki tumors relative to KHT-C tumors. However, in both cases, tumors with higher initial IFP values showed enhanced survival following treatment with ZD6126 ($P = 0.003$ for KHT-C; $P < 0.0001$ for CaSki). Despite the differences in baseline cell survival between the two tumors, there was a similar variation in survival of about two orders of magnitude from the lowest to the highest IFP values. There was no difference in clonogenic survival in the control tumors as a function of pretreatment IFP.

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**Figure 3.** Blood flow in a representative KHT-C tumor before, during, and shortly after treatment with ZD6126. Blood flow was measured using DOCT. Y axis, normalized pixel count with Doppler signals above the image noise level within the DOCT field of view (2 mm wide × 1.5 mm deep). This measure of the flowing blood volume fraction was calculated from each of the DOCT images acquired sequentially over the 40-minute imaging interval. Dashed line, drug injection time.

**Figure 4.** Oxygen levels over time in representative control and ZD6126-treated KHT-C tumors. $p_O2$ levels were measured using the Oxylite 4000 system.
Elevated tumor IFP has been linked to reduced drug delivery and efficacy in experimental studies (6–12) and to poor prognosis in patients (3, 13). In this study, we sought to elucidate the effects of a vascular disrupting agent, ZD6126, on tumor IFP, and conversely the effect of tumor IFP on response to treatment with ZD6126.

Initial studies were carried out using the KHT-C murine fibrosarcoma tumor. This tumor has been extensively studied within our laboratory, and in other laboratories, including use in studies with ZD6126; the drug doses used in this study were based on data obtained from previous work (27). Both drug doses (100 and 200 mg/kg) were found to significantly decrease tumor IFP levels, resulting in an ~75% reduction in tumor IFP at 72 hours post-treatment. However, there were temporal fluctuations in IFP following treatment with ZD6126 (Fig. 1A), with an initial decrease at 1 hour followed by recovery at 3 hours to near pretreatment levels and a later second decline. These fluctuations presumably reflect differences in the balance of physiologic factors that together determine the magnitude of IFP in individual tumors. Our results, showing differences in IFP as a function of time, are consistent with variable results of vascular disrupting agents on IFP reported by others who examined one specific time point (24, 40).

It is likely that the vascular hydraulic permeability-surface area product is significantly greater than the interstitial hydraulic conductivity in most untreated tumors so that IFP tracks capillary pressure (32, 41). Variation in IFP among tumors therefore likely reflects differences in the underlying precapillary and capillary blood flow resistance (33, 41–44). Hori et al. (24) showed rapid, reversible constriction of precapillary tumor arterioles following administration of a combretastatin derivative, followed by permanent central vascular congestion and thrombosis by 3 hours. Our IFP observations are compatible with these results: Arteriolar constriction would be expected to increase precapillary blood flow resistance and reduce IFP, whereas the later development of capillary thrombosis should increase capillary blood flow resistance and restore IFP toward its initial value. Both of these changes would contribute to reduced blood flow. The later second decline in IFP at times beyond 24 hours probably reflects the development of central tumor necrosis (27). Obliteration of the central tumor vasculature, leaving only a rim of malignant cells perfused by more normal host vessels (22, 30, 31), would permanently reduce the vascular hydraulic permeability-surface area product, blood flow resistance, and IFP. Antiangiogenic strategies that inhibit VEGF have also shown sustained reductions in IFP attributable to regression of abnormal vessels (6). Higher interstitial hydraulic conductivity as a result of necrosis might also contribute to lower IFP at late times.

The temporal fluctuations in IFP described here for the KHT-C tumor were examined in another tumor model, the human cervical carcinoma xenograft CaSki. The IFP changes were similar over time, with the exception that the CaSki tumors displayed a continuous decline in IFP without the recovery at 3 hours seen with KHT-C (Fig. 1B). The reduction in IFP at 1 hour following ZD6126 was less than that seen in the KHT-C tumors and the subsequent decline in IFP was more gradual. It is likely that the difference between the two tumor types is due to the relative balance among the underlying factors that determine IFP.

**Table 1. Thickness of the viable cell rim in KHT tumors at 24 and 48 hours posttreatment with ZD6126 (six tumors per group)**

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<th>Time posttreatment (h)</th>
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<th>Mean rim thickness (no. cells)</th>
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<td>Mean ± SE, 25 ± 1.18</td>
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example, it is possible that the reversible effect of ZD6126 on arteriolar endothelial cells described by Hori et al. (24) occurs more slowly or is more prolonged in CaSki, in relation to the time course over which capillary thrombosis develops. Alternatively, it may be that CaSki tumor cells are more reliant on a supply of oxygen and other nutrients and thus tumor necrosis occurs more rapidly when this supply is interrupted. Taken together, the KHT-C and CaSki results illustrate the complex relationship that exists between IFP and blood flow, and provide additional evidence that IFP is a minimally invasive marker of vascular “normality” in tumors (45).

To further explore the mechanics of the antivascular affects of ZD6126, and to show a response of the drug similar to that reported previously by others, a series of experiments designed to measure tumor blood flow, hypoxia, and necrosis were carried out in the KHT-C tumor model. DOCT imaging technology was used to monitor blood flow over a 30-minute time period following administration of ZD6126. The results showed clear cessation of blood flow within 20 minutes of treatment, providing strong evidence for rapid vascular damage (Fig. 3). The time span over which capillary thrombosis develops. Alternatively, it may be that the drug elicits a degree of tumor cell toxicity in vivo, its effect may be subject to the problem that occurs with other chemotherapeutic agents, namely that drug diffusion into the tumor is poor and thus it is rendered less effective in high IFP tumors. However, in vitro studies gave no evidence that the drug was toxic to the tumor cells at concentrations similar to those expected in vivo (data not shown).

We found no difference between low and high IFP tumors in the thickness of the rim of cells that persisted 24 hours after ZD6126 (Table 1). It follows that, regardless of the precise mechanism to explain the relationship between IFP and response to ZD6126, the greater number of surviving cells (approximately two orders of magnitude) in high versus low IFP tumors may not entirely be confined to the rim. Beauregard et al. (40) showed regional heterogeneity in the vascular response to combretastatin measured using dynamic contrast-enhanced MRI in keeping with this possibility.

Overall, this study has shown that tumor IFP is reduced by treatment with the vascular disrupting agent ZD6126 in both the KHT-C and CaSki tumor models. Although the temporal fluctuations between the two tumor types differ, the overall result is the same. Equally, both tumor types showed a link between high tumor IFP and improved cell survival following treatment with ZD6126. Although these two tumor models have intrinsic biological and molecular differences and were grown in different recipient hosts (immune deficient SCID mice for CaSki and immune-competent C3H mice for KHT-C), together these data add support to the increasing evidence that designates elevated tumor IFP as a negative factor in cancer therapy. The fact that the vascular disrupting agent ZD6126 is able to reduce IFP suggests that if such agents are used appropriately in conjunction with other drugs, the efficacy of treatment may be enhanced as shown in recent work showing improved response in tumor models (46).

Acknowledgments
Grant support: National Cancer Institute (C) and Terry Fox Foundation with funds raised from the Terry Fox Run.

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We thank Bob Kuba and Ami Syed for expert technical assistance, Dr. Kärstein Maasèide for advice on the microscopy and statistics, and Dr. Anderson Ryan (AstraZeneca, Macclesfield, United Kingdom) for providing the ZD6126.


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