Pharmacodynamic-mediated effects of the angiogenesis inhibitor SU5416 on the tumor disposition of temozolomide in subcutaneous and intracerebral glioma xenograft models

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Running Title Page

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d) Abbreviations: C_p = plasma concentrations; C_t = tumor interstitial fluid unbound concentrations; ss = steady-state; VEGF = vascular endothelial cell growth factor; VEGFR2 = vascular endothelial cell growth factor receptor two

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ABSTRACT

The objective of this study was to determine the tumor distribution of temozolomide, an alkylating agent, in the absence and presence of the angiogenesis inhibitor, SU5416, a specific VEGFR2 inhibitor. The study was conducted in nude rats bearing either subcutaneous or intracerebral tumors that overexpressed VEGF. For both tumor locations, animals were assigned to either of two treatment groups, SU5416 (25 mg/kg, dissolved in dimethylsulfoxide) or vehicle control, dimethylsulfoxide, (710 µl/kg) administered IP every day for a total of 9 doses. Twenty-four hours after the last dose of SU5416 or dimethylsulfoxide, temozolomide was administered as a steady-state infusion regimen designed to achieve target plasma concentrations (C_p) of 20 µg/ml. In addition to the measurement of temozolomide C_p, tumor interstitial fluid unbound concentrations (C_t) of temozolomide were evaluated by microdialysis.

In subcutaneous tumors, SU5416 treatment produced a 24% reduction in steady-state temozolomide C_t values (p < 0.05) as well as 21% reductions in tumor/plasma concentration ratios (C_t / C_p; p = 0.11) compared to controls. In intracerebral tumors, steady-state temozolomide C_t and C_t / C_p ratios were significantly increased by 2-fold in the SU5416 treatment group compared to control. The apparent paradoxical effect of SU5416 on the tumor disposition of temozolomide in subcutaneous and intracerebral tumors is discussed in the context of physiological changes (ex. interstitial fluid pressure, microvessel density) and the sampling region in the tumor. It is proposed that the net balance of antiangiogenic drug-mediated
pharmacodynamic actions will determine how drug disposition in tumors may be affected.

The ability to combine anticancer drugs into effective therapeutic regimens is a cornerstone of successful cancer chemotherapy. A common theme is to combine agents with different mechanisms of action and nonoverlapping toxicities as a means to improve the therapeutic index. The combination of angiogenesis inhibitors and cytotoxic agents represent two classes of drugs that exemplify this latter dogma of combining drugs with different pharmacological targets and different toxicities. Antiangiogenic drugs inhibit endothelial cell proliferation and neovascularization by various mechanisms, with many of the newer agents able to interrupt different growth factor pathways. Cytotoxic drugs target tumor cells and cause cell death through a diverse range of mechanisms, and most often possess a dose-limiting toxicity of myelosuppression. In preclinical models the combination of angiogenesis inhibitors and cytotoxic drugs compared to single agent therapy have shown increased efficacy based on endpoints of tumor growth (Kato et al., 1994; Teicher et al., 1996; Cascinu et al., 1999; Browder et al., 2000). Except for our previous investigations (Devineni et al., 1996; Ma et al., 2001), there have been no pharmacokinetic investigations of combinations of antiangiogenic and cytotoxic drugs.

The growth and metastatic nature of tumors are intimately dependent on the process of angiogenesis. This multi-step process may be characterized by the angiogenic phenotype, which includes the breakdown of capillaries and a state of
vascular hyperpermeability. We had hypothesized that successful antiangiogenic therapy would reverse the state of vascular hyperpermeability leading to a normal vascular phenotype that would be less permeable to the transport of low molecular weight anticancer drugs. In support of this proposal, we showed TNP-470, an antiangiogenic compound, caused significant reductions in the tumor concentrations of temozolomide, an alkylating agent (Devineni et al., 1996; Ma et al., 2001). The reductions in temozolomide concentrations were found in both subcutaneous and intracerebral gliomas that overexpressed vascular endothelial cell growth factor [VEGF], a key angiogenic factor, but not in isogeneic tumors with low VEGF expression (Ma et al., 2001). Since it was found that microvessel density in tumors also decreased in the TNP-470 treatment groups, the reductions in temozolomide concentrations were attributed to TNP-470’s action on vascular density and permability. In addition, because TNP-470 and temozolomide did not interact pharmacokinetically, the reduced tumor concentrations of temozolomide were attributed to the pharmacodynamic actions of TNP-470 on the tumor vasculature.

The current investigation was designed to extend our evaluation of interactions between angiogenesis inhibitors and cytotoxic drugs by use of another angiogenesis inhibitor, SU5416, a specific receptor tyrosine kinase inhibitor of VEGFR2 located on endothelial cells (Fong et al., 1999; Mendel et al., 2000; Smolich et al., 2001). Evaluation of the SU5416:temozolomide interaction was conducted in nude rats bearing either subcutaneous or intracerebral tumors that overexpressed VEGF (V+) (Ma et al., 1998), and incorporated microdialysis to assess tumor interstitial fluid temozolomide concentrations, the analogous model used in the TNP-
METHODS

Materials

Temozolomide and SU5416 were kindly provided by Schering-Plough Research Institute (Kenilworth, NJ), and Sugan Pharmaceutical Company (South San Francisco, CA), respectively. Soft-tissue microdialysis probes (CMA\20), brain microdialysis probes (CMA\12), a refrigerated fraction collector (CMA\170), and a Freely Moving Animal restraint system were purchased from CMA Microdialysis (N. Chelmsford, MA). Male adult nude rats (bw: 170 - 240 g) were purchased from Taconic Farms, Inc. (Germantown, NY). HPLC analyses were performed with a Hewlett Packard Model 1050 liquid chromatographic system (Sunnyvale, CA). All other chemicals and supplies were obtained from commercial sources.

Tumor Model and Implantation

Our previously established human glioma cell line SF188/V+ was used throughout the investigations (Ma et al., 1998). This model is based on the parental human SF188 cell line which had been found to have low VEGF expression. A subline, SF188/V+ or V+, that overexpressed VEGF was derived from SF188 cells by transfection with the mouse full length VEGF_{164} cDNA as reported previously (Ma et al., 1998). Cells were grown as monolayers in DMEM medium containing 10% FCS and were maintained in a humidified atmosphere of 5% CO₂ in air at 37°C.
All animal protocols were approved by the IUPAC in accordance with NIH guidelines. For the *in vivo* subcutaneous glioma model, male nude rats were briefly anesthetized with ether, and had implanted approximately $5 \times 10^6$ V+ cells suspended in 0.4 ml of Matrigel (Collaborative Biomedical, Bedford, MA), subcutaneously in the dorsal neck region. After cell implantation, animals were returned to their cages and received standard rat diet and water *ad libitum*. Animal body weight and tumor size were measured regularly throughout the study periods. Tumor volume was calculated as; tumor volume (mm$^3$) = $0.5ab^2$, where $a$ is the longest diameter and $b$ is the shortest diameter.

For the intracerebral tumor model, rats were anesthetized with an intraperitoneal dose (0.1 ml/100 g body weight) of a 3:2:1 (v:v:v) mixture of ketamine hydrochloride (100 mg/ml): acepromazine maleate (10 mg/ml): xylazine hydrochloride (20 mg/ml), secured in a stereotaxic apparatus, and had implanted 3 $\mu$l of a tumor cell suspension ($10^8$ cells/ml) into the right thalamic region as previously described (Devineni et al., 1996; Ma et al., 2001). A microdialysis guide cannula was inserted at the same location at a depth of 3 mm, and then cemented into place prior to suturing the skin. The animals were then returned to the cages and received standard rat diet and water *ad libitum*. Animal body weight was measured every day throughout the study periods.

**Pharmacokinetic Studies**

SU5416 treatment
A SU5416 dosage regimen of 25 mg/kg IP daily X 9 dissolved in dimethylsulfoxide was used throughout the investigation. This schedule was shown to be effective in other tumor models (Fong et al., 1999; Mendel et al., 2000) as well as in preliminary studies conducted by us. Initiation of SU5416 (25 mg/kg IP daily X 9 dissolved in dimethylsulfoxide) or vehicle control (dimethylsulfoxide, 710 µl/kg) therapy was based on two different criteria depending on whether animals bore a subcutaneous or intracerebral tumor. When subcutaneous tumors had grown to approximately 12 mm in the longest dimension SU5416 or control treatment commenced. In the intracerebral study, either SU5416 or vehicle control treatment began once the animals had an approximate 10 g loss of body weight maintained for two consecutive days. The weight loss was normally accompanied by the appearance of CNS symptoms [i.e. unsteady gait, arched back] related to the tumor. Vehicle control and SU5416 treated animals received the same volume of dimethylsulfoxide (710 µl/kg).

Surgical Procedures, Microdialysis, and Temozolomide Dosing

On the last day of SU5416 or vehicle control treatment (Day 9), a right common carotid artery and a jugular vein cannula were implanted for temozolomide administration and blood sampling, respectively.

Tumor microdialysis was conducted in both subcutaneous and intracerebral tumors to provide a measure of interstitial fluid unbound or free temozolomide concentrations by the zero-flow microdialysis calibration method (Chaurasia, 1999). On the day of temozolomide administration (one day following the last dose of
SU5416 or vehicle), one microdialysis probe was inserted into the peripheral region of each subcutaneous tumor and perfused with Ringer’s solution at 4 µl/min for at least 45 min before the administration of temozolomide. For each intracerebral tumor, a brain microdialysis probe was inserted into the guide cannula and perfused with simulated cerebrospinal fluid (1.1 mM MgCl₂, 1.35 mM CaCl₂·H₂O, 3 mM KCl, 0.242 mM NaHPO₄·7H₂O, 20 mM NaHCO₃, and 131.9 mM NaCl) at 4 µl/min for at least 45 min before administration of temozolomide. Temozolomide was then given intraarterially to achieve steady-state plasma concentrations of 20 µg/ml for at least 7 hours by infusing 2 mg/kg/min over 10 min, followed by a 7 hour infusion at 0.2 mg/kg/min of temozolomide. During the 7 hour temozolomide infusions, the microdialysis flowrate was varied from 4, 1, 3, 2, and 6 µl/min consistent with the zero-flow calibration method (Chaurasia, 1999). At each flowrate from 4 to 6 serial dialysate samples were collected in individual vials containing 3.7 µl – 5.0 µl of 1 N HCl to preserve the chemical stability of temozolomide. The dialysate samples were stored at −80°C until analyzed by HPLC as described below. Multiple plasma samples (13 samples/animal) were collected during the 7 hour temozolomide infusions, and then stored at −80°C until analysed by HPLC.

The zero-flow microdialysis calibration method is conducted under steady-state temozolomide plasma concentrations in conjunction with variable microdialysis flowrates. It is known that drug recovery across the dialysis membrane and the associated dialysate drug concentrations are a function of the dialysate flow rate, which is used as the independent variable in the exponential equation characterizing the change in temozolomide dialysate concentrations as a function of flow rate. The
equation assumes that other parameters \([i.e.]\) dialysis membrane permeability and surface area] are constant during the experiment. The equation is fit to the measured temozolomide dialysate concentrations to obtain an estimate of the zero-flow or actual interstitial fluid concentration. Temozolomide dialysate concentrations collected from the last two fractions \([\text{approximately } 20 – 30 \text{ min periods}]\) at each flow rate were used in the estimation of the zero-flow concentration (Chaurasia, 1999).

**HPLC Analysis of Temozolomide**

Plasma \((100 \, \mu l)\) was acidified with \(20 \, \mu l\) of \(1\text{N} \text{HCl}\) and then \(200 \, \mu l\) of cold acetonitrile \((\text{ACN})\) was added to precipitate proteins. The tubes were vortexed and centrifuged at \(15,000 \text{ rpm for } 5 \text{ min.}\) The resulting supernatant \((100 \, \mu l)\) was combined with \(100 \, \mu l\) of mobile phase that consisted of \(5\% \, (v/v) \text{ACN in } 0.05 \text{ M ammonium acetate buffer, pH } 6.8.\) The mixture was vortexed and \(10 \, \mu l\) of the sample injected onto the HPLC system. Dialysate samples \((10 \, \mu l)\) were injected directly onto the HPLC system which consisted of a CN column \((150 \, \text{mm} \times 4.6 \, \text{mm, } 5 \, \mu m; \text{Alltech Spherisorb}),\) and UV detector. Temozolomide was detected at \(323 \text{ nm}\) at a flowrate of \(0.7 \text{ ml/min.}\) An external standard calibration method was used to calculate plasma and dialysate temozolomide concentrations. The HPLC assay was accurate and precise with coefficients of variation of \(15\%\) or less.

**Immunohistochemical staining for CD31**
A standard immunohistochemical assay was used to measure microvessel density (Ma et al., 2001; Pietras et al., 2001). Vascular endothelial cells were stained with an anti-CD31 monoclonal antibody (TLD-3A12, Research Diagnostics Inc, Franders NJ) on 5 µm paraffin-embedded sections. Tissue sections incubated without the primary antibody were used as negative controls. After immunostaining, microvessel density in subcutaneous tumors was measured in three different tumor areas including the tumor:stromal interface. In the intracerebral study, microvessel density was evaluated in at least 5 different regions. Microvessel density was quantitated by image analysis (Adobe Photoshop) that measured pixel intensity based on a digital selection criteria corresponding to the stained endothelial cells. The percentage of microvessel density in each section was obtained by dividing the intensity of the stained endothelial cells by the total pixel intensity in the same microscopic field.

**Statistical analyses**

Comparisons between the dimethylsulfoxide vehicle control and SU5416 treatment groups were made for temozolomide plasma and tumor concentrations, tumor/plasma concentration ratios, and microvessel density using an ANOVA test (JMP, version 5). Statistical significance was indicated by values of p < 0.05.

**RESULTS**
Pharmacokinetic Studies

Two basic types of study designs were available to obtain pharmacokinetic information to evaluate the potential of a drug interaction between SU5416 and temozolomide. In one design, temozolomide concentrations are collected under non-steady-state conditions, typically following intravascular bolus administrations. The second design utilizes a temozolomide administration regimen that produces steady-state drug concentrations. Each design enables an assessment of temozolomide distribution into tumors under control and SU5416 treatment conditions. Under non-steady-state conditions, tumor uptake can be assessed by the ratio of the area under-the drug concentration-time curve \([\text{AUC}]\) in tumor divided by the AUC in plasma, whereas under steady-state conditions the ratio of steady-state tumor to plasma drug concentrations provides an analogous assessment. The current investigation utilized the steady-state study design because the zero-flow microdialysis calibration method, that is conducted under steady-state conditions, is less prone to calibration errors associated with methods used for non-steady-state conditions, such as retrodialysis.

Subcutaneous Tumor Study

In both the dimethylsulfoxide vehicle control and SU5416 treatment groups, steady-state temozolomide plasma concentrations were achieved, and were 22.2 ± 4.2 µg/ml and 21.0 ± 3.0 µg/ml respectively (Figure 1A). Table 1 shows the physiological and temozolomide pharmacokinetic parameters from the tumor microdialysis study. SU5416 decreased tumor size compared to vehicle control by
about 24%. The steady-state tumor interstitial fluid unbound temozolomide concentrations were 16.5 ± 3.7 µg/ml, and 12.6 ± 3.9 µg/ml in control and SU5416 treatment groups, respectively. This 24% reduction in temozolomide tumor concentrations by SU5416 was significant when compared to dimethylsulfoxide control (p=0.038), yet the difference in the steady-state Ct / Cp temozolomide concentration ratios did not quite reach statistical significance (p = 0.11), being 0.77 ± 0.23 and 0.61 ± 0.23 in control and SU5416 treatment groups, respectively. The Ct / Cp concentration ratios reflect differences in the actual steady-state plasma concentrations, and provide the most definitive assessment of differences in drug distribution between the two treatment groups. Thus, the reduction in temozolomide’s steady-state distribution into tumors due to SU5416 was not significantly different than the dimethylsulfoxide control treatment.

Intracerebral Tumor Study

Investigation of drug disposition in tumors is readily accomplished when tumors are located subcutaneously because of their defined boundaries and easy access. These features minimize the chance of contamination by normal tissue, and ensure that the measured drug concentrations are, in fact, tumoral. Animals tolerate subcutaneous tumors well, and pharmacokinetic studies can be accomplished without significant morbidity or mortality, factors that complicate performing similar studies in animals bearing intracerebral tumors. At the same time, pharmacokinetic studies performed in intracerebral tumor models yield a more mechanistic and clinically-relevant characterization of drug disposition because the tumor is located in
the normal tissue of origin. Drug transport obstacles presented by the blood-brain barrier and blood-tumor barrier are more likely to resemble the human situation.

Table 2 shows the physiological and temozolomide pharmacokinetic parameters from the intracerebral study. Temozolomide plasma concentrations in the SU5416 and vehicle control groups were similar (p > 0.05), being 19.2 ± 0.5 µg/ml, and 19.3 ± 2.14 µg/ml, respectively (Figure 1B). The steady-state temozolomide tumor interstitial concentrations were significantly increased (p < 0.05) in the SU5416 treatment group (5.3 ± 2.6 µg/ml) compared to the control group (2.8 ± 1.2 µg/ml). Accordingly, the steady-state temozolomide C_t / C_p ratio increased 100% from 0.14 ± 0.05 in the control group to 0.28 ± 0.12 (p < 0.05) in the SU5416 group (Table 2 and Figure 2). This increased temozolomide tumor to plasma concentration ratio is in contrast to that observed in subcutaneous tumors under analogous drug treatment protocols. Possible explanations for the findings are given in the Discussion.

**Microvessel density analysis**

A number of biological endpoints are being proposed as pharmacodynamic indicies of antiangiogenic therapy (Jain et al., 1997). Amongst these parameters, microvessel density has been consistently measured based on immunohistochemical methods using different endothelial cell markers. The anti-CD31 method is relatively common and shown to be an accurate measure of neovascularization (Giatromanolaki et al., 1997). In subcutaneous tumors, three different regions were used for the analysis; two peripheral regions of the tumor and the stroma, defined as a 100 µm wide area at the leading edge of the tumor. In the
intracerebral group, microvessel density was quantitated in at least 5 different sites in the tumor. In most cases the highest microvessel density was within 100 µm of the leading edge of the tumor. There were significant reductions in microvessel density in subcutaneous tumors in the stroma region in SU5416 treated animals compared to control (p < 0.05). There were also reductions in microvessel density in the peripheral regions of subcutaneous tumors (control mean = 37.44 ± 12.74 %; SU5416 mean = 28.33 ± 10.98 %; p > 0.05), however this reduction due to SU5416 treatment did not reach statistical significance. Intracerebral tumors exhibited about a 16% reduction of tumor microvessel density (control mean = 8.4 ± 3.0 %; SU5416 mean = 7.1 ± 1.8 %; p > 0.05) in the SU5416 treated group compared to the control when averaged over all sampled regions. Table 3 provides the microvessel density [mean ± SD] measurements for each region and treatment group.

Tumor necrosis could readily be evaluated in subcutaneous tumors since they were easily accessible. Intracerebral tumors were hard to collect as discrete samples because they were smaller, interdigitated with normal brain and were analyzed for microdialysis probe placement. For subcutaneous tumors, 62% of tumors showed greater than 50% necrosis in the dimethylsulfoxide control, whereas in the SU5416 treatment group only 13% of the tumors showed greater than 50% necrosis.

**DISCUSSION**
Combination chemotherapy is an important strategy in the treatment of cancer. The ability to combine drugs with different mechanisms of action, and non-overlapping toxicities may enhance efficacy and attenuate the development of drug resistance. In many instances the design of combination dosing regimens is arrived at semi-empirically without detailed pharmacokinetic and pharmacodynamic analyses. Rarely are measurements of drug concentrations in tumor used as a basis to evaluate combination anticancer drug therapy. In consideration of the pharmacodynamic basis of angiogenesis inhibitor and cytotoxic drug interactions, we have emphasized the importance of determining drug concentration measurements in tumors. Our previous work in this area has shown that the angiogenesis inhibitor, TNP-470, reduced tumor concentrations of temozolomide in both syngenic and xenograft glioma models (Devineni et al, 1996; Ma et al., 2001), that were attributed to a reduction in microvessel density and capillary permeability caused by TNP-470.

In light of the current study, which found increased temozolomide brain-tumor concentrations following SU5416 treatment, a reanalysis of the mechanisms responsible for interactions between angiogenesis inhibitors and cytotoxic drugs was required.

In all of our investigations we have utilized microdialysis to obtain a measure of unbound temozolomide concentrations in the interstitial fluid of tumors. Microdialysis samples collected from subcutaneous V+ tumors were from the tumor periphery, the area most indicative of antiangiogenic drug action because it is the area of active neovascularization. In intracerebral V+ tumors, stereotaxic implantation of the microdialysis guide cannulas at the time of tumor cell implantation does not permit
selection of central or peripheral sampling. In addition, due to the irregular and invasive growth pattern of gliomas, it is difficult to categorize the microdialysis samples as representing either central or peripheral regions of the tumor. At the termination of the pharmacokinetic studies, gross pathological examinations of whole brains were undertaken to ensure that the microdialysis probes were in the tumor; yet it is not possible to categorize whether the probe is located in either a central or peripheral region. Thus, temozolomide tumor concentrations collected by microdialysis from subcutaneous tumors can be designated as peripheral, whereas those from intracerebral tumors can not as readily be designated. Since tumors cannot be assumed to be homogeneous compartments with respect to drug concentrations, and physiological parameters, such as blood flow and interstitial fluid pressure, it is useful to consider regional differences in these variables. This is particularly important in the assessment of drug interactions with angiogenesis inhibitors as they can affect many physiological variables. Therefore, the effects of antiangiogenic therapy on temozolomide tumor concentrations could depend on whether the microdialysis samples were collected from either the peripheral or central region of the tumor.

Assessment of the effects of antiangiogenic therapy on drug disposition in tumors is based on changes relative to the vehicle control. In the series of TNP-470 studies an aqueous (1% ethanol, 5% guar gum in normal saline) vehicle was used (Ma et al., 2001), whereas for the SU5416 studies dimethylsulfoxide was utilized as the vehicle control because of its ability to solubilize SU5416. Some interesting differences were found that were not anticipated because all other experimental conditions were
equivalent in the control groups. Steady-state temozolomide brain-tumor concentrations and tumor/plasma ratios were much less in the dimethylsulfoxide control group \((2.77 \pm 1.20 \, \mu g/ml, \frac{C_t}{C_p} = 0.14)\) compared to the aqueous control group used in the TNP-470 study \((8.6 \pm 2.1 \, \mu g/ml, \frac{C_t}{C_p} = 0.39)\). Examination of subcutaneous tumors in the dimethylsulfoxide control group revealed appreciable tumor necrosis that was not observed in the TNP-470 aqueous control group. Therefore, to fully contrast the TNP-470:temozolomide and SU5416:temozolomide investigations, consideration of both the control vehicles and regional differences in drug concentrations is required.

In the TNP-470:temozolomide studies, regardless of the tumor location or microdialysis sampling site, TNP-470 treatment always decreased temozolomide tumor concentrations. The reductions in the steady-state \(\frac{C_t}{C_p}\) temozolomide concentration ratios were 30% and 50% in the subcutaneous and intracerebral V+ tumors, respectively (Ma et al., 2001). These reductions were associated with nearly analogous reductions of 30% and 63% in microvessel density in the subcutaneous and intracerebral V+ tumors, respectively (Ma et al., 2001), and supported our previous assertion that antiangiogenic therapy decreased cytotoxic drug concentrations by inhibition of capillary density and permeability.

In the SU5416:temozolomide studies, a differential effect of SU5416 was observed on tumor concentrations of temozolomide. In subcutaneous V+ tumors, the action of SU5416 caused a 21% reduction in steady-state tumor/plasma temozolomide concentration ratios. Even though this reduction did not achieve statistical significance, the effect was similar to that of TNP-470, and was also accompanied by
a 20-35% reduction in microvessel density. An opposite action of SU5416 was found in intracerebral tumors with steady-state \( \frac{C_t}{C_p} \) temozolomide concentration ratios increased by 100% compared to control. As indicated above, the dimethylsulfoxide control treatment group attained much lower temozolomide brain-tumor concentrations than the aqueous control in the TNP-470:temozolomide study. This was not the case in subcutaneous tumors in which both the dimethylsulfoxide and aqueous vehicle control groups attained mean temozolomide interstitial fluid concentrations and \( \frac{C_t}{C_p} \) ratios of about 16 ug/ml and 0.75, respectively. Since microdialysis sampling in the subcutaneous tumors was in the peripheral region, the potential necrotic action of dimethylsulfoxide may have been minimized as necrosis normally emanates from central regions of the tumor. The much lower temozolomide brain-tumor concentrations observed in the dimethylsulfoxide control group would suggest that microdialysis samples were from central necrotic regions that may have been amplified by dimethylsulfoxide. Tumor necrosis is indicative of hypoxia and elevated interstitial fluid pressure due to the lack of integrity in the capillary network. Recently, it has been proposed that antiangiogenic therapy can normalize the tumor vasculature and improve the delivery of therapeutic agents (Jain, 2001). This normalization is associated with a reduction in interstitial fluid pressure and hypoxia. In our study, SU5416 may have restored or normalized the capillary architecture, decreasing interstitial fluid pressure and hypoxia that yielded increased temozolomide brain-tumor concentrations. This normalization effect on the tumor vasculature was not apparent in subcutaneous V+ tumors since microdialysis sampling was at the periphery, an area less prone to necrosis and hypoxia.
Therefore, the differential action of SU5416 had on temozolomide subcutaneous and intracerebral tumor concentrations is attributed to the microdialysis sampling site, peripheral versus central, and the dimethylsulfoxide administration vehicle.

Pivotal work by Jain (Jain, 1987; Jain, 1988; Jain, 1990) has shown that the physiological variables of organ blood flow, interstitial fluid pressure, pH and hypoxia are heterogeneous within tumors and can impact on macromolecule transport. Consistent with the aforementioned “normalization” effect, a number of investigations have shown angiogenesis inhibitors can alter tumor blood flow, hypoxia and interstitial fluid pressure as well as microvessel density (Dres et al., 2000; Lee et al., 2000; Pietras et al., 2001). It was shown that the tumor uptake of a low molecular weight marker, $^{51}$Cr-EDTA, was increased in the presence of the PDGF receptor kinase inhibitor, STI571, presumably due to the latter’s ability to reduce interstitial fluid pressure (Pietras et al., 2001). These data are consistent with our proposal, in that SU5416, through restoration of the capillary archetecture, may have decreased interstitial fluid pressure and enhanced temozolomide’s brain-tumor concentrations. It should also be appreciated that even though dimethylsulfoxide may have contributed to tumor necrosis, the normal progression of tumor growth and metastasis does lead to hypoxic and necrotic tumors. Thus, the positive action of SU5416 on temozolomide’s brain-tumor concentrations may also occur in tumors containing hypoxic regions as a result of common growth patterns of solid tumors.

In conclusion, we have demonstrated that the angiogenesis inhibitor, SU5416, resulted in either increased [brain-tumor] or decreased [subcutaneous] temozolomide tumor concentrations. It is believed that this differential result is due to
the microdialysis sampling site, central vs. peripheral, that may have been enhanced by the ability of dimethylsulfoxide to cause tumor necrosis. It is proposed that angiogenesis inhibitors can alter multiple tumor physiological variables (i.e. microvessel density, permeability, hypoxia, interstitial fluid pressure) that can result in pharmacodynamic-mediated changes in tumor drug concentrations. The ultimate effect on cytotoxic drug concentrations will be determined by the net balance of physiological effects that depend on the tumor and the local environment in which drug concentrations are measured. The continued evaluation of angiogenesis inhibitor:cytotoxic drug interactions should incorporate independent assessments of the multiple physiological variables to fully understand the mechanisms underlying changes in drug concentrations in tumors.
REFERENCES


FOOTNOTES

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Legends for Figures

Figure 1. Mean [± SD] steady-state temozolomide [TMZ] plasma concentrations in dimethylsulfoxide control and SU5416 treatment groups in subcutaneous tumor study (A, n = 7) and intracerebral tumor study (B, n = 6).

Figure 2. Steady-state plasma and tumor interstitial fluid unbound temozolomide [TMZ] concentrations (left Y-axis) and the tumor interstitial fluid unbound:plasma temozolomide concentration ratios (right Y-axis) in animals bearing V+ intracerebral tumors. Animals received either dimethylsulfoxide vehicle control (C) or 25 mg/kg SU5416 (S) IP for 9 daily doses. N= 6 in each treatment group. Some symbols overlap.
Table 1. Physiological and temozolomide pharmacokinetic parameters from the subcutaneous tumor microdialysis study.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Tumor size (mm$^3$) Pre-treatment</th>
<th>Tumor size (mm$^3$) Post-treatment</th>
<th>ss $C_p$ ($\mu$g/ml)</th>
<th>ss $C_t$ ($\mu$g/ml)</th>
<th>ss $C_t / C_p$</th>
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</thead>
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<tr>
<td>dimethylsulfoxide vehicle</td>
<td>1386 ± 631$^a$</td>
<td>1684 ± 873</td>
<td>22.2 ± 4.2$^b$</td>
<td>16.5 ± 3.7$^c$</td>
<td>0.77 ± 0.23$^b$</td>
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<tr>
<td>SU5416 (25 mg/kg)</td>
<td>1345 ± 591</td>
<td>1315 ± 546</td>
<td>21.0 ± 2.2</td>
<td>12.6 ± 3.9</td>
<td>0.61 ± 0.23</td>
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</table>

$^a$ ss $C_p$: steady-state plasma concentrations; ss $C_t$: steady-state interstitial fluid tumor concentrations. All values represent mean ± SD, n = 7.

$^b$ p > 0.05 compared to SU5416 treatment group.

$^c$ p < 0.05 compared to SU5416 treatment group.
Table 2. Physiological and temozolomide pharmacokinetic parameters from the intracerebral tumor microdialysis study.

<table>
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<tr>
<th>Treatment groups</th>
<th>Parameters&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ss C&lt;sub&gt;p&lt;/sub&gt; (ug/ml)</th>
<th>ss C&lt;sub&gt;t&lt;/sub&gt; (ug/ml)</th>
<th>ss C&lt;sub&gt;t&lt;/sub&gt; / C&lt;sub&gt;p&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>dimethylsulfoxide vehicle</td>
<td></td>
<td>19.3 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.8 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>SU5416 (25 mg/kg)</td>
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<td>19.2 ± 4.1</td>
<td>5.3 ± 2.6</td>
<td>0.28 ± 0.12</td>
</tr>
</tbody>
</table>

<sup>a</sup> ss C<sub>p</sub>: steady-state plasma concentrations; ss C<sub>t</sub>: steady-state interstitial fluid tumor concentrations. All values represent mean ± SD, n = 6.

<sup>b</sup> p > 0.05 compared to SU5416 treatment group.

<sup>c</sup> p < 0.05 compared to SU5416 treatment group.
Table 3. Microvessel density in subcutaneous (SC) and intracerebral (IC) tumors following treatment regimens of either SU5416 or dimethylsulfoxide vehicle in microdialysis study.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Periphery/SC</td>
</tr>
<tr>
<td>dimethylsulfoxide vehicle</td>
<td>37.4 ± 12.7</td>
</tr>
<tr>
<td>SU5416 (25 mg/kg)</td>
<td>28.3 ± 11.0</td>
</tr>
</tbody>
</table>

*all values are expressed as a percentage and represent the mean ± SD, n = 6.

* p > 0.05 compared to SU5416 treatment group.

* p < 0.05 compared to SU5416 treatment group.
Figure 1

A

B