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ORIGINAL ARTICLE

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Tumor selective antivascular effects of the novel antimitotic compound ABT-751: an in vivo rat regional hemodynamic study

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Abstract Selective induction of vascular damage within a growing tumor is a potentially important approach in the search for potent anticancer therapeutics. Tubulin-binding (antimitotic) agents destabilize cellular microtubules, suppress tumor growth, and exert antivascular effects with varying degrees of tumor selectivity in preclinical models. The tumor-selective, antivascular effects of ABT-751, a novel, orally active antimitotic agent, currently in phase II clinical development, were characterized in vivo in the present study. We developed an in vivo rat model designed to quantify acute changes in regional vascular resistance (VR) in both tumor and non-tumor vascular beds simultaneously. Tissue-isolated tumors (1 g) with blood flow supplied by a single epigastric artery were grown in rats. Subsequently, tumor blood flow was measured under anesthesia in solid tumors and also in mesenteric, renal, and normal epigastric arteries. Phenylephrine-induced (1 μmol/kg) increases in VR were not different between tumor and non-tumor epigastric arteries, suggesting that tumor vessels possess relatively normal vasoconstrictive function. ABT-751 (3, 10, and 30 mg/kg; i.v.) produced modest transient increases in mean arterial pressure with no effect on heart rate. Tumor VR increased to 75 ± 36, 732 ± 172, and $727 \pm 125\%$ above baseline, respectively ($P < 0.05$ for the 10 and 30 mg/kg doses), whereas VR in normal epigastric arteries was not significantly affected. Administration of ABT-751 produced transient modest ($P < 0.05$) increases in mesenteric VR and no effect on renal VR. These results demonstrate that ABT-751 produces marked reductions in tumor blood flow in the intact rat at doses that exert negligible effects on normal vascular function.

Keywords Tumor · Blood flow · Tubulin · ABT-751 · In vivo · Rat

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Introduction

Solid tumors are dependent upon a functional vascular network for survival and continued growth, making tumor blood vessels a logical target for therapeutic intervention. There are two primary approaches to targeting the tumor vasculature as a means to inhibit tumor growth. One is the prevention of new vessel growth (angiogenesis), a highly active area of research [8]. Another is the selective inhibition of vascular function, leading to vascular collapse within a tumor as a means to diminish tumor perfusion, an effect that should also suppress tumor growth and increase cell death.

Tubulin-destabilizing agents, such as colchicine and the vinca alkaloids vincristine and vinblastine, have been shown to reduce tumor blood flow and to produce marked tumor necrosis [1, 5, 14], albeit at doses approaching the maximally tolerated dose (MTD) [13]. Thus, while it is clear that the antivascular effects of these cytotoxic compounds may contribute to their ability to suppress tumor growth, the inability to separate tumor vascular effects from the MTD has generally limited their use as vascular targeting agents. These observations have led to the discovery of other tubulin-binding agents such as combretastatin A4 phosphate (CA-4P) and ZD6126 that exert tumor-selective, antivascular effects at nontoxic doses [4, 6]. Currently, both CA-4P and ZD6126 are in clinical development as tumor selective, antivascular agents [7, 21, 23]. ABT-751 (E7010) is a colchicine-binding agent

originally discovered by Eisai Company, and is a novel antiproliferative sulfonamide [27, 28] with potent tubulin-destabilizing activity [19]. This compound is currently in clinical development.

The mechanism by which tubulin-destabilizing compounds such as CA-4P and ZD6126 derive their antivascular effect and apparent tumor selectivity have not been fully elucidated. As demonstrated in vitro and in vivo, alterations in endothelial cell function may play a role in addition to the unique structural properties of the neovasculature [2, 6, 18, 25]. While effects on endothelial cell shape and function are likely important, it should be noted that the vasoconstrictive effects of tubulin disruption does not require an intact endothelium, suggesting a direct effect on vascular smooth muscle [20].

Results from phase I trials indicate that both CA-4P and ZD6126 reduce tumor blood flow when measured via MRI [23]. However, even though both agents possess reasonable tolerability profiles, recent preliminary reports of phase I studies have indicated CA-4P and ZD6126 may inhibit normal vascular function at doses targeted to inhibit tumor perfusion. CA-4P has been associated with coronary constriction [7] and constriction of irradiated bowel [21], while ZD6126 has been reported to alter arterial pressure [23]. Thus, these reports suggest that the therapeutic index of tubulin-binding agents may be less than previously considered. Therefore, we designed a preclinical in vivo model to characterize the simultaneous effects of ABT-751 on tumor and non-tumor vascular function.

In this report, we describe the in vivo regional hemodynamic effects of ABT-751 in rats bearing a tissue-isolated tumor with a single artery and vein. Importantly, we characterized the simultaneous and dose-dependent effects of ABT-751 on vascular resistance in tumors with a comparison to that of epigastric, mesentery, and kidney. These results demonstrate that administration of ABT-751 causes a selective, dose-dependent reduction in tumor blood flow, with corresponding increases in tumor vascular resistance while exerting little to no effect on the normal vascular beds studied. Thus, the present results demonstrate that in addition to its antiproliferative effects, ABT-751 exerts tumor-selective, antivascular effects in the rat. Whether the tumor-selective profile of ABT-751 occurs in the clinical setting remains to be determined.

Materials and methods

Animals

Male Fisher 344 rats (250–300 g; Charles River, Portage, Mich.) were housed individually in a temperature-controlled room and provided with water and food ad libitum (Harlan:Teclad 8640 rodent diet). All experiments were conducted in accordance with the National Institutes of Health guidelines for the use of experimental animals and were approved by the Abbott Laboratories Institutional Animal Care and Use Committee.

Silicone chambers

Spherical silicone chambers were constructed from MDX4-4210 Medical Grade Silicone Elastomer (Factor II, Lakeside, Ariz.). The silicone was applied to Teflon spheres (12 mm diameter) and cured at 210°F for 10 min. Once cool, the silicone was removed from the Teflon sphere, yielding a hollow chamber with an internal volume of approximately 1 ml. The silicone chambers were stored under aseptic conditions until implantation.

Isolated tumor preparation

Rat 9L glioma cells (American Type Culture Collection, Manassas, Va.) were grown to confluency using standard cell culture techniques in Dulbecco's modified Eagle's medium supplemented (1:1; v:v) with F12 nutrient mixture plus 10% fetal calf serum and an antibiotic–antimycotic solution containing streptomycin sulfate/penicillin G/amphotericin B at 200 IU/ml. Once confluent, cells were harvested and suspended in sterile saline at 5×10^6 cells/ml. Rats were sedated using CO_2, the abdomen shaved and 0.1 ml of the cell suspension was injected subcutaneously into the lower abdomen using a 26-gauge needle. Once ambulatory, the rats were returned to general housing. After 7–10 days, the subcutaneous abdominal tumors (approximately 0.3 g) were aseptically removed from the animals and prepared for reimplantation by removal of all connective tissue and cut into 0.2-g cubes.

The method for growing indwelling inguinal tumors supplied by a single artery and vein has been previously described by Tozer et al. [24]. Briefly, naive, recipient rats were anesthetized with ketamine 90 mg/kg (Fort Dodge Animal Health, Fort Dodge, Iowa) and xylazine 10 mg/kg (Phoenix Pharmaceuticals, St Joseph, Mo.) and the inguinal area of the left hind limb prepared for aseptic surgical implantation. Via an inguinal incision the superficial epigastric artery and vein of the left hind limb were exposed and dissected free of adventitia, except for approximately 0.2 g of the inguinal fat pad immediately attached to the epigastric artery and vein. A single tumor fragment was attached to the fat pad using 5-0 silk sutures. The tumor tissue and fat pad were secured inside the silicone chamber using a small amount of tissue adhesive (Vetbond; 3M Animal Care Products, St Paul, Minn.). The chamber was then secured within the inguinal cleft to the abdominal wall with additional tissue adhesive and filled with sterile saline. The skin was closed over the chamber and secured with wound clips. Animals were allowed to recover on a warming pad until sternally recumbent, then returned to general housing.

After 14–17 days the animals were anesthetized with inactin 100 mg/kg i.p. (Sigma, St Louis, Mo.) and the growth of the encapsulated tumors was assessed. The long-acting barbiturate, inactin (thiobutabarbital), was chosen for these studies because of its minimal effects on cardiovascular tone [3] and its ability to provide a sustained, stable plane of surgical anesthesia in the rat. These properties contribute to the use of Inactin as an anesthetic of choice for rodent hemodynamic studies [22]. Animals with tumors of sufficient size (approximately 1 g) still possessing the single artery and vein were accepted for hemodynamic evaluation and instrumented as described below.

Regional hemodynamic assessment of normal and tumor vascular beds

Inactin-anesthetized rats were intubated (PE-240) via a small tracheostomy. A catheter (PE-50, Becton Dickinson, Sparks, Md.) was placed in the right carotid artery for measurement of mean arterial pressure (MAP) and heart rate (HR). Two additional catheters (PE-50) were implanted in a single jugular vein to allow for continuous infusion of isotonic saline to maintain hydration (10 μl/min) and for administration of drug. ABT-751 was synthesized at Abbott Laboratories (Abbott Park, Ill.) and was dissolved in a vehicle of PEG200/5% dextrose (1:1) in water (Sigma/Abbott Laboratories).

A pulsed Doppler flow cuff (Triton Technology, San Diego, Calif.), size 0.8 mm, was positioned on the single artery supplying the encapsulated tumor. Additional Doppler flow cuffs were positioned on the mesenteric artery (1.3 mm), renal artery (0.8 mm) and the contralateral superficial epigastric artery (0.8 mm) of the non-tumor-bearing leg [12]. The flow cuffs were connected to a pulsed Doppler flow meter (Triton Technology) and the output signal in turn fed to a DC amplifier (Modular Instruments, Southeastern, Pa.). Pulsatile blood flow (BF) was recorded digitally using a PONEMAH Data Acquisition Physiology Platform System (Gould Instrument Systems, Valley View, Ohio); MAP, HR, and blood flow measurements were sampled every 5 s and condensed into 1-min averages. MAP and HR were derived from the carotid artery pressure waveform by PONEMAH software.

Following instrumentation, the animals were maintained on a warming pad and allowed to stabilize for at least 1 h. To minimize dehydration from anesthesia and surgical stress, isotonic saline was infused continuously throughout the protocol at 10 μl/min. After a 60-min stabilization period, a 15-min baseline period was established for all recorded parameters. Subsequently, ABT-751 was infused for 15 min and the animals monitored for 2 h after treatment. Blood and tumor tissue samples were collected at the end of the experimental protocol for measurement of plasma and tissue drug concentrations (Department of Exploratory Kinetics, Abbott Laboratories).

Analysis of ABT-751 concentration in plasma and tumor tissue

A single liquid–liquid extraction was used to separate ABT-751 from plasma or tumor homogenate. A plasma aliquot (0.2 ml sample or spiked standard) was combined with an equal volume of an internal standard and 6 ml ethyl acetate/hexane (90:10 or 50:50, v/v). The samples were vortexed vigorously for 30 s followed by centrifugation. The organic layer was transferred to a clean glass centrifuge tube and evaporated to dryness with a gentle stream of dry air at room temperature. The samples were reconstituted by vortexing with a mixture of acetonitrile and 1% acetic acid in water (40:60, v/v). ABT-751 and the internal standard were separated from endogenous contaminants on a 5 or 10 cm × 3.0 mm Kromasil C_{18} column (Keystone), with an acetonitrile/1% aqueous acetic acid mobile phase (50:50, v/v) at a flow rate of 0.4–0.5 ml/min with a 20 μl sample injection volume. Analysis was performed on a Sciex API-III+ or API3000 biomolecular mass analyzer with a heated nebulizer interface. Analytes were ionized in the positive ion mode with a source temperature of approximately 480 K. Detection was in the multiple reaction monitoring (MRM) mode at m/z 372.0 → 200.1 for ABT-751 and m/z 368.0 → 136.3 for the internal standard. ABT-751 and internal standard peak areas were determined using Sciex MacQuan software. The method, evaluated over the concentration range from 0 to 6664 ng/ml, was linear (correlation coefficient >0.99), with a variability generally $<6\%$ (%CV) from the analysis of triplicate standards at seven concentrations in mouse, rat, dog, and monkey plasma. The limit of quantitation was about 2 ng/ml from a 0.2-ml plasma sample using MS/MS detection. Similar sensitivity and reproducibility were obtained for the analysis of tumor samples.

Data and statistical analysis

All data are represented as mean ± SEM. For systemic and regional hemodynamic variables, data were collected at 1-min intervals then averaged into 15-min time values. Regional vascular resistance values were calculated as MAP/BF. Peak vascular resistance values were limited to a maximum of 1000% to avoid achieving "infinite" resistance values that occur as a result of blood flow falling to nearly undetectable levels. The 1000% cutoff reflects resistance values obtained at the lowest measurable blood flow values (approximately 0.5–1 kHz shift). Changes in MAP, HR and regional vascular resistance are expressed as percent change from baseline (time zero).

Dose-response functions of phenylephrine (PE) were analyzed by repeated measures analysis of variance (ANOVA) followed by the Newman–Keuls post hoc test. Differences in vascular resistance between normal tissue and tumor were analyzed by Student's t-test (paired). Differences between the vehicle control group

and the ABT-751-treated groups were determined using an ANOVA with a Dunnett's post hoc at every 15-min time point. Significance was set at $P < 0.05$ for all comparisons.

Results

Systemic and regional hemodynamic effects of phenylephrine

Consistent with the known effects of systemic administration of an α_1-adrenergic agonist, PE produced dose-dependent increases in MAP and HR concomitant with increased vascular resistance (Table 1). Importantly, PE-induced increases in regional vascular resistance were not significantly different between normal epigastric and tumor epigastric vascular beds ($226 \pm 78\%$ vs $363 \pm 144\%$ increases, respectively; Fig. 1), thereby demonstrating functional similarity between normal and tumor vascular beds.

Table 1 Effects of phenylephrine administration on MAP and HR in tumor-bearing rats ($n = 7$). The data presented are percent change from baseline

	Saline	Phenylephrine (nmol/kg)			Recovery
		100	300	1000	
MAP	2.7 ± 1.2	14.2 ± 3.2*	24.5 ± 4.0*	29.3 ± 6.5*	16.6 ± 4.1*
HR	0.2 ± 0.4	9.5 ± 2.6*	17.5 ± 4.1*	19.6 ± 4.7*	15 ± 3.3*

*$P < 0.05$ compared to baseline.

Systemic and regional hemodynamic effects of ABT-751

Figure 2 shows waveforms of arterial pressure and regional blood flows obtained in the four vascular beds in the same animal during baseline, and 60 min following administration of ABT-751 at 30 mg/kg. These waveforms demonstrate both the pulsatile nature of the blood flow to the tumor under basal conditions, and more importantly, the nearly complete abolition of tumor blood flow produced by ABT-751 compared to the normal vascular beds in the same animal.

Infusion of ABT-751 at 3 and 30 mg/kg had no effect on MAP compared to the vehicle-treated group. ABT-751 at 10 mg/kg produced a modest dose-dependent increase in MAP with a response duration of less than 30 min. In spite of the increase in MAP produced by the 10 mg/kg dose, ABT-751 produced no significant effects on HR at any dose tested (Fig. 3).

Concomitant with elevations in MAP, administration of ABT-751 at 10 and 30 mg/kg produced modest increases in mesenteric vascular resistance. These responses were dose-dependent, and exhibited a 30-min duration of action. In contrast to mesenteric vascular resistance, renal vascular resistance was not significantly affected by ABT-751 at any dose tested (Fig. 4).

The effects of ABT-751 on tumor vascular resistance and epigastric vascular resistance in the contralateral, non-tumor bearing leg are summarized in Fig. 5. ABT-751 at 3 mg/kg had no effect on tumor vascular resistance, similar to its lack of effect on MAP and vascular resistance in non-tumor vascular beds.

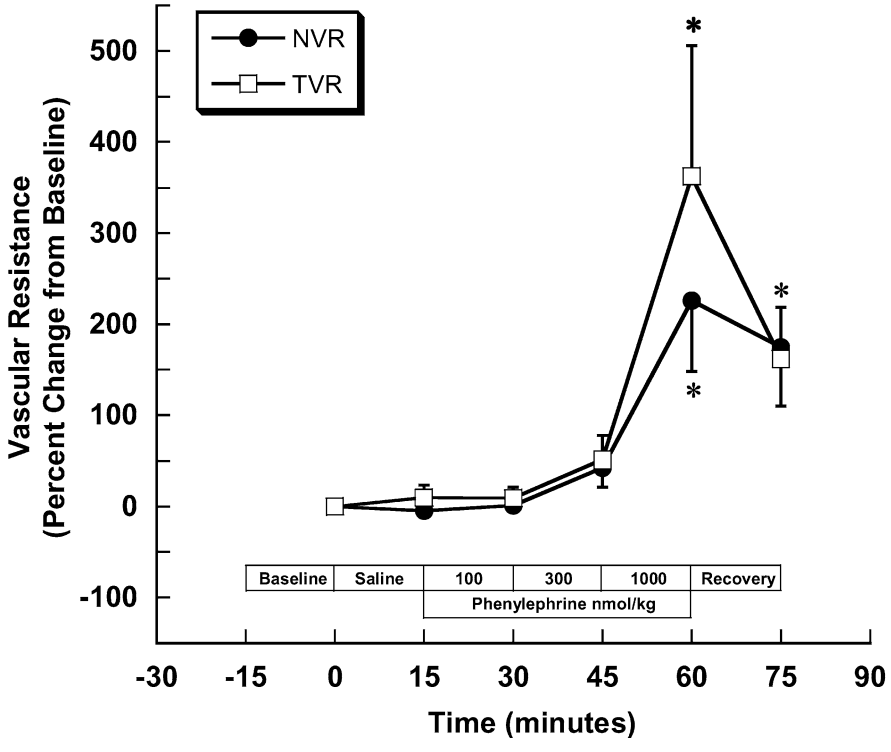

Fig. 1 Effects of saline and phenylephrine on normal epigastric and tumor vascular resistance in rats bearing an isolated tumor supplied by a single epigastric artery and vein. Values are mean ± SEM percent change from baseline ($n = 7$) (*NVR*, *closed circles* normal epigastric vascular resistance; *TVR*, *open squares* tumor vascular resistance). *$P < 0.05$ vs baseline

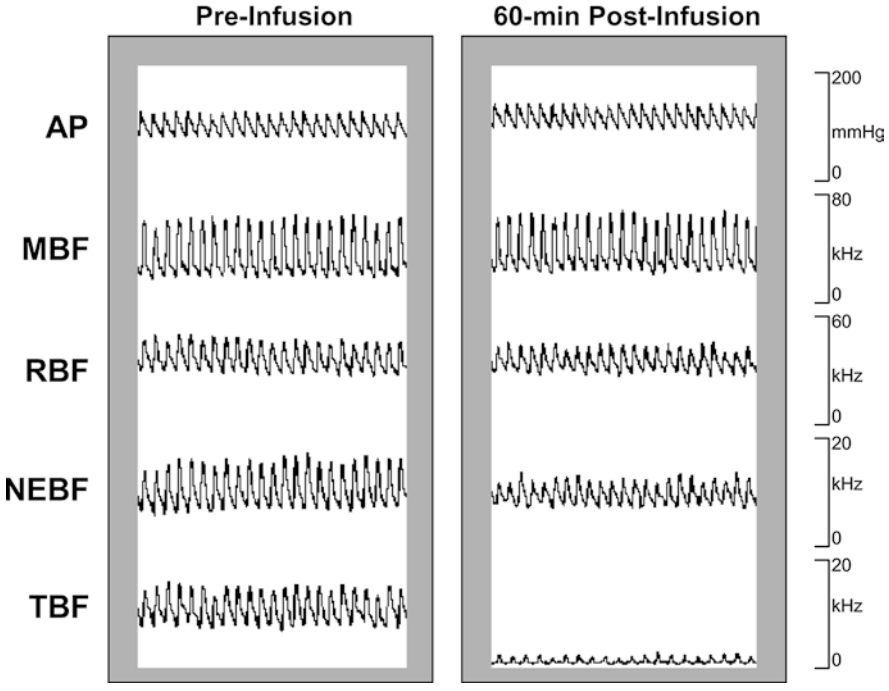

Fig. 2 Representative pulsatile waveforms from a rat bearing a tumor supplied by a single artery and vein both before ABT-751 (*left panel*) and 60-min after dosing at 30 mg/kg (*right panel*) (*AP* arterial pressure, *HR* heart rate, *MBF* mesenteric blood flow, *RBF* renal blood flow, *NEBF* normal epigastric blood flow, *TBF* tumor blood flow)

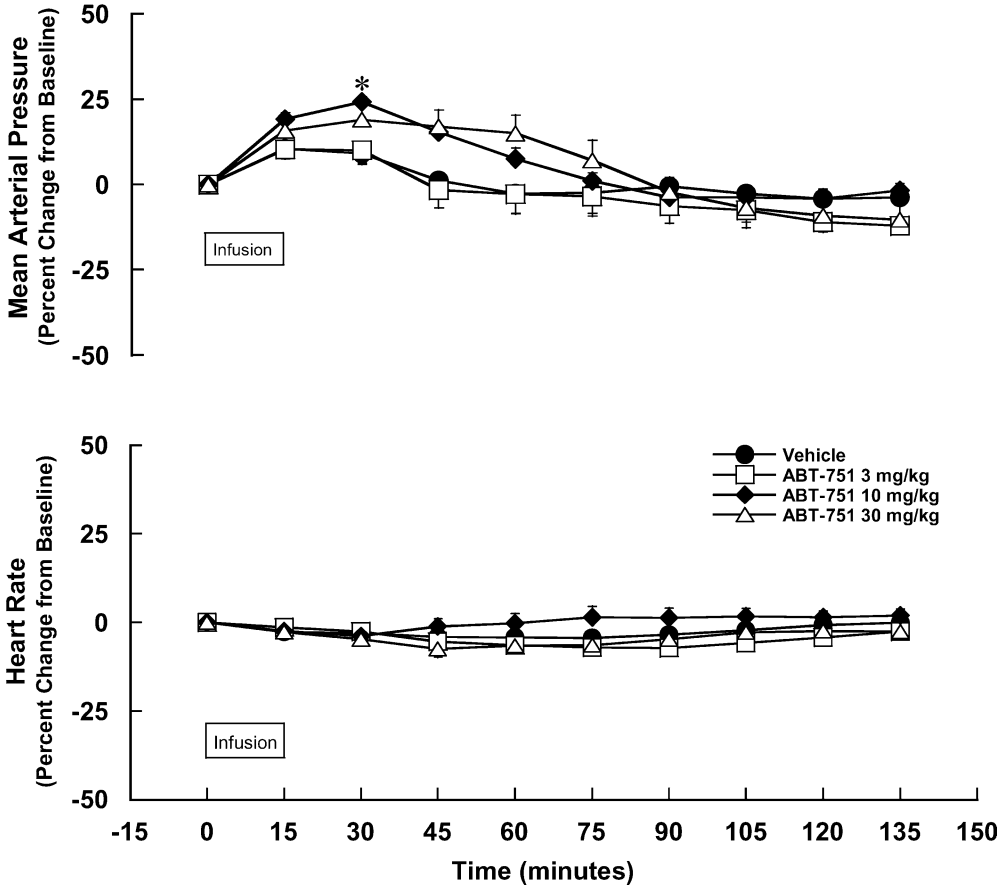

Fig. 3 Effects of ABT-751 on MAP and HR in rats bearing an isolated tumor supplied by a single epigastric artery and vein. Immediately following baseline, ABT-751 was administered over 15 min in a volume of 2 μl/g. Values are mean ± SEM percent change from baseline ($n = 4$–6). *$P < 0.05$ vs vehicle

At 10 and 30 mg/kg, ABT-751 produced rapid, dose-dependent increases in tumor vascular resistance. By the end of the 15-min infusion period, tumor vascular resistance had increased by 89 ± 17% and 53 ± 16% above baseline in response to the 10 and 30 mg/kg doses, respectively. Subsequently, at 15 min after treatment, tumor vascular resistance had increased to 623 ± 100% and 520 ± 145% above baseline, respectively, with peak

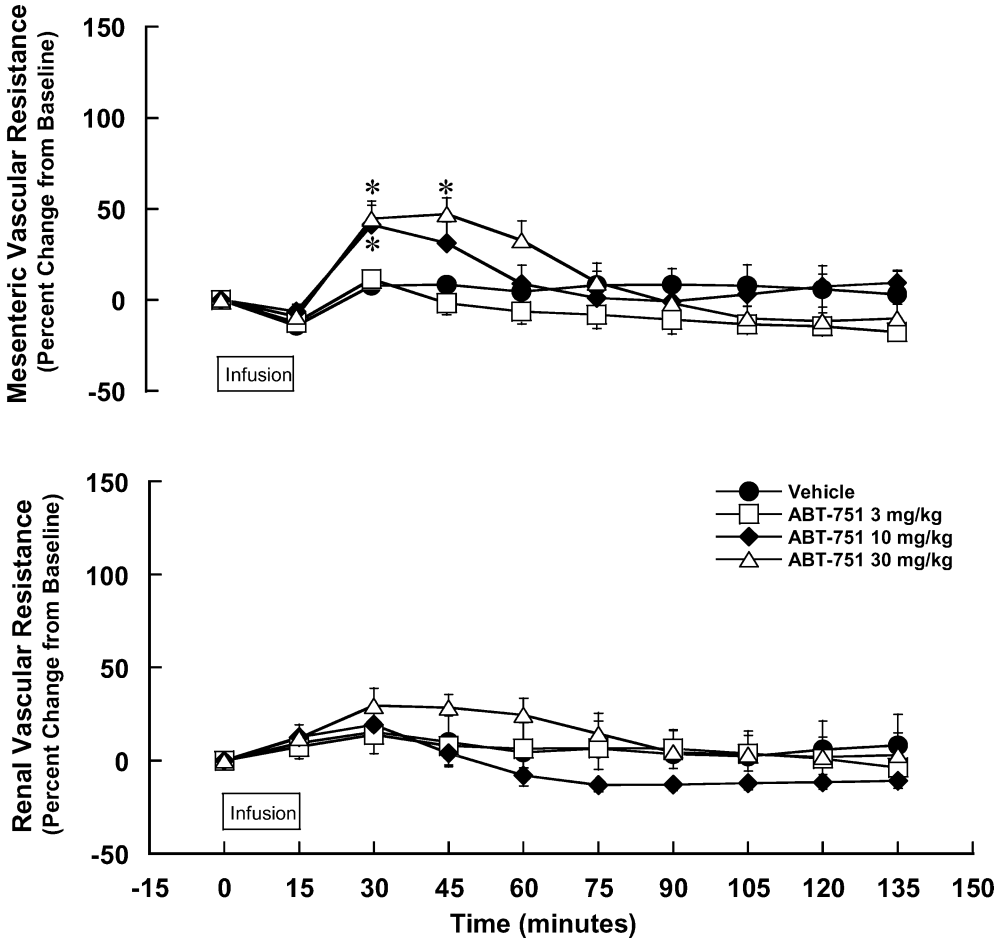

Fig. 4 Effects of ABT-751 on mesenteric and renal vascular resistance in rats bearing an isolated tumor supplied by a single epigastric artery and vein. Immediately following baseline, ABT-751 was administered over 15 min in a volume of 2 μl/g. Values are mean ± SEM percent change from baseline ($n = 4$–6). *$P < 0.05$ vs vehicle

increases of 732 ± 172% and 727 ± 125% above baseline occurring 45 min after the infusion was terminated. In rats receiving the high dose of ABT-751, tumor vascular resistance remained significantly elevated above baseline throughout the remainder of the experimental protocol.

In contrast to its ability to markedly increase tumor vascular resistance, ABT-751 produced no significant change in epigastric vascular resistance in the normal, contralateral leg (Fig. 5). Moreover, tumor vascular resistance remained significantly elevated throughout the entire 2-h observation period in response to the 30 mg/kg dose even though MAP and vascular resistance in mesenteric, renal and epigastric vascular beds were not different from baseline at 45 min after treatment.

At the end of the 2-h observation period, ABT-751 concentrations were threefold to fourfold higher in the tumor tissue than in plasma (Table 2).

Discussion

Using an in vivo isolated tumor model, we demonstrated that: (1) ABT-751 (E7010) effectively decreased blood flow to a solid tumor by increasing tumor vascular resistance; (2) the increase in tumor vascular resistance was dose-dependent both in magnitude and duration; and (3) the action of ABT-751 was selective for tumors, reducing tumor blood flow while exerting minimal to no effect on normal vascular beds. To our knowledge, these results characterize for the first time in the intact animal the concentration- and time-dependent antivascular effects of a potent antimitotic agent (ABT-751) at doses that abolish tumor blood flow while producing little functional effect in non-tumor vascular beds.

ABT-751 is a novel orally active sulfonamide currently in phase II clinical development as an antimitotic agent. Preclinically, ABT-751 has demonstrated inhibition of tubulin polymerization with an IC_{50} value of 3.1 μM [10, 17], and binds preferentially to the β_3 isotype of tubulin with a K_i of 3.3 μM in a competitive colchicine-binding assay [15, 17, 27]. In addition, ABT-751 dose-dependently inhibits cellular proliferation, and increases the mitotic index in vitro of human tumor cell lines [27, 28]. When administered orally, ABT-751 demonstrates good efficacy against rodent tumors and a wide range of human tumor xenografts including lung, gastric, colon and breast cancers [9, 16].

In the present study, we combined the measurement of regional hemodynamic function in an anesthetized rat with a "tissue isolated" tumor model, in which tumors are grown in vivo with an existing single artery and vein

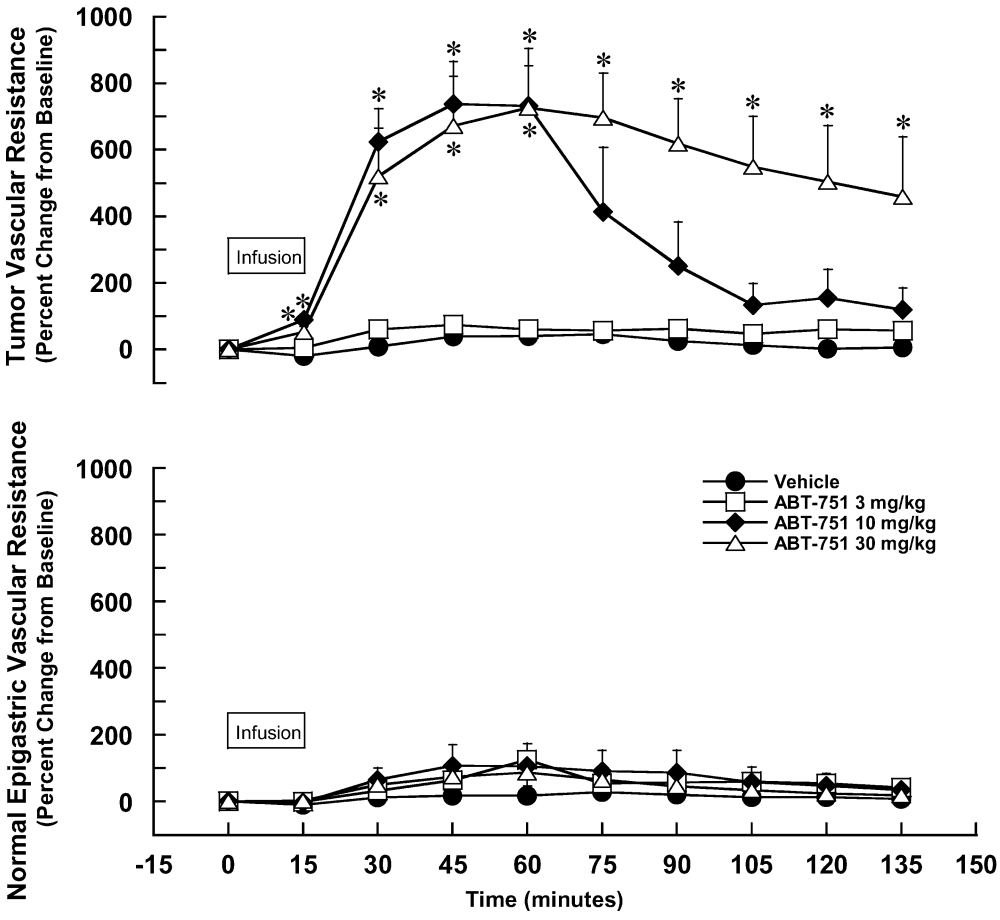

Fig. 5 Effects of ABT-751 on normal epigastric (contralateral leg) and tumor vascular resistance in rats bearing an isolated tumor supplied by a single epigastric artery and vein. Immediately following baseline, ABT-751 was administered over 15 min in a volume of 2 μl/g. Note the change in scale vs Fig. 4. Values are mean ± SEM percent change from baseline ($n = 4$–6). *$P < 0.05$ vs vehicle

Table 2 Concentration and ratio of ABT-751 in tumor and plasma. Values are means ± SEM

ABT-751 dose (mg/kg)	Tumor (μg/g)	Plasma (μg/ml)	Tumor/plasma ratio	*n*
3	0.3 ± 0.1	0.1 ± 0.02	2.8 ± 0.9	5
10	1.4 ± 0.7	0.4 ± 0.1	3.9 ± 2.3	4
30	3.7 ± 0.4	1.2 ± 0.2	3.4 ± 0.6	6

[24], thus allowing us to measure the real-time hemodynamic profile of ABT-751 in tumor and non-tumor tissues. It is important to note that these were not transplanted vessels, but rather the tumor blood supply was derived from a preexisting epigastric artery.

As shown in Fig. 1, the dose-dependent vasoconstrictive effects of PE were similar for tumors and the contralateral, non-tumor-bearing epigastric arteries. Thus, these results suggest that under the conditions of the present study, the tumor vasculature possessed relatively normal contractile function. Moreover, treatment-induced reductions in tumor blood flow were not associated with constriction of the single epigastric artery that supplied blood to the tumor or normal tissue. Therefore, measured reductions in tumor blood flow reflected vascular events occurring distal to the flow probe and within the tumor itself, not the conduit vessels.

Administration of ABT-751 to the anesthetized tumor-bearing rat produced modest, transient elevations in MAP concomitant with modest elevations in mesenteric vascular resistance and dose-dependent reductions in tumor blood flow. Since HR remained unchanged, these findings suggest that the transient increase in MAP produced by ABT-751 is mediated by increases in peripheral vascular resistance, such as those observed in the mesenteric vasculature. More substantial increases in blood pressure have been reported for another tubulin-destabilizing agent, CA-4P, at doses (100 mg/kg, i.p.) that increased regional vascular resistance values at 1 h after dosing, and produced near cessation of tumor blood flow at 6 h after dosing, as measured using radiolabeled iodoantipyrine [25]. In a more recent study in anesthetized tumor-bearing rats acutely instrumented for intravital microscopy, lower doses of CA-4P (30 mg/kg, i.p.) increased MAP to more than 125% above control levels while decreasing red blood cell velocity in tumor surface capillaries and venules. A higher dose (100 mg/kg, i.p.) produced even greater increases in blood pressure, and further reductions in perfusion of tumor surface vessels [26]. Thus, in preclinical models, CA-4P appears to reduce indices of tumor perfusion at doses that increase arterial pressure, and constrict normal vascular beds, suggesting that a limited

therapeutic index may exist for the compound's anti-vascular effects.

ABT-751 produced rapid, marked increases in tumor vascular resistance while producing limited effects in the normal, non-tumor vascular beds studied. Elevations in tumor vascular resistance produced by ABT-751 were dose-dependent both in magnitude of response and duration of action. In the high-dose group, ABT-751 decreased tumor blood flow to nearly undetectable levels. Subsequently, tumor vascular resistance tended to decrease modestly throughout the 2-h post-treatment period, a response that likely occurred in parallel to reductions in plasma and tissue concentrations.

In the present study, ABT-751 plasma concentrations at the end of the 2-h observation period averaged 0.1 ± 0.02, 0.4 ± 0.1, 1.2 ± 0.2 μg/ml for the 3, 10, and 30 mg/kg doses, respectively (Table 2). Tumor concentrations of ABT-751 were threefold to fourfold above circulating plasma levels following the 2-h observation period (Table 2). This difference could have been due to increased binding of the molecule to microtubulin in the highly permeable tumor vasculature, or could simply reflect reduced washout of ABT-751 secondary to blood flow. Pharmacokinetic studies using a similar infusion protocol with more frequent blood sampling provided peak concentrations (Cmax) of 4.6 and 15.9 μg/ml for 3 and 10 mg/kg doses. The peak concentrations in the 3 and 10 mg/kg dose groups are very similar to those obtained in human clinical trials with ABT-751 [11]. Following once-daily 200 or 300 mg oral doses for 7 days in adult patients with solid tumors, peak concentrations averaged 6.6 ± 1.7 and 13.1 ± 2.3 μg/ml, respectively. A 21-day schedule with the 200 mg dose provided slightly higher peak concentrations of 9.4 ± 2.9 μg/ml. Thus, the plasma concentrations achieved in the present study are similar to those observed clinically.

Compounds such as ZD6126 and CA-4P are also currently undergoing clinical investigation [7, 21, 23]. ZD6126 delays tumor growth in a variety of human xenograft models [2], and decreases tumor functional vascular volume in tumor-bearing mice [6]. In addition, ZD6126 causes shut-down of newly formed capillaries in mice in the Matrigel plug assay, an effect that is reversible after 22 h [18]. However, consistent with effects on normal vasculature, preliminary reports of phase I clinical trials indicate that ZD6126 alters blood pressure [23] and that CA-4P is associated with coronary constriction [7] and constriction of irradiated bowel [21]. Therefore, the apparent effects of these compounds on normal vasculature may limit therapeutic utility.

In summary, the present study demonstrated that ABT-751, a novel tubulin-binding agent, produces marked reductions in tumor blood flow in the intact rat at doses that exert negligible effects on normal vascular function. Ongoing clinical studies will determine whether tumor selective, antivascular effects of ABT-751 are achievable under clinical conditions.

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