Effect of the Multitargeted Receptor Tyrosine Kinase Inhibitor, ABT-869 \[N-(4-(3-amino-1H-indazol-4-yl)phenyl)-N’-(2-fluoro-5-methylphenyl)urea\], on Blood Pressure in Conscious Rats and Mice: Reversal with Antihypertensive Agents and Effect on Tumor Growth Inhibition


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ABSTRACT

ABT-869 \[N-(4-(3-amino-1H-indazol-4-yl)phenyl)-N’-(2-fluoro-5-methylphenyl)urea\] is a novel multitargeted inhibitor of the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptor tyrosine kinase family members. ABT-869 demonstrates tumor growth inhibition in multiple preclinical animal models and in early clinical trials. VEGF receptor inhibition is also associated with reversible hypertension that may limit its benefit clinically. To evaluate optimal therapeutic approaches to prevent hypertension with VEGF receptor inhibition, we characterized the dose-dependent effects of seven antihypertensive agents from three mechanistic classes [angiotensin-converting enzyme inhibitors (ACEis), angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs)] on hypertension induced by ABT-869 in conscious telemetry rats. We report that ABT-869-induced hypertension can be prevented and reversed with subtherapeutic or therapeutic doses of antihypertensive drugs with a general rank order of ACEi > ARB > CCB. In SCID mice, the ACE inhibitor, enalapril \((C_{20}H_{28}N_2O_5)\) at 30 mg/kg, prevented hypertension, with no attenuation of the antitumor efficacy of ABT-869. These studies demonstrate that the adverse cardiovascular effects of the VEGF/PDGF receptor tyrosine kinase inhibitor, ABT-869, are readily controlled by conventional antihypertensive therapy without affecting antitumor efficacy.

The formation of new blood vessels from the pre-existing vasculature, referred to as angiogenesis, is a fundamental step in the creation of new blood supplies supporting tumor growth and is also a mechanism whereby tumor cells move from the primary tumor site into circulation (Zetter, 1998). It is now well established that receptor tyrosine kinases (RTKs) and their growth factor ligands play a fundamental role in tumor angiogenesis (Blume-Jensen and Hunter, 2001). Studies in knockout mice demonstrated that VEGF signaling through VEGFR2 (also referred to as FLK1 or KDR) and FMS-like tyrosine kinase 1 are necessary for embryo survival and normal vasculature (Gschwind et al., 2004). Thus, many subsequent studies point to the importance of the VEGF signaling complex in tumor growth and angiogenesis, and a number of important studies have demonstrated the antitumor efficacy of agents targeting these receptors. It has been shown that anti-VEGF antibodies suppress the growth of tumor xenografts in nude mice (Kim et al., 1993), and studies performed in the same model have shown that dominant-negative VEGFR2 mutants block the growth of subcutaneously implanted glioblastomas (Millauer et al., 1994). Moreover, a humanized monoclonal antibody to VEGF,
bevacizumab, was recently approved for the treatment of colorectal cancer patients through the inhibition of angiogenesis (Gschwind et al., 2004).

Small-molecule VEGF receptor blockers, such as ABT-869 (Fig. 1), have demonstrated antitumor activity (Tomayko and Reynolds, 1989; Yoshiji et al., 2001; Schenone et al., 2007). However, the kinase selectivity panel of ABT-869 extends beyond that of a single protein; ABT-869 is a novel multitargeted inhibitor of multiple VEGF and PDGF receptor tyrosine kinase family members and has been shown to exhibit significant tumor growth inhibition in multiple preclinical animal models (Albert et al., 2006; Dai et al., 2007; Shankar et al., 2007; Zhou et al., 2008a,b). Moreover, it has been suggested that simultaneous inhibition of the VEGF and PDGF RTKs, which intercede the advancement of tumors through different mechanisms, may result in greater antitumor efficacy and has the promise of treating a larger range of human cancers than more selective VEGF inhibitors (Albert et al., 2006).

Although VEGF-targeted chemotherapeutic agents may have substantial therapeutic benefit to cancer patients (Faivre et al., 2006), mechanism-based hypertension represents a potential hurdle for this class of compounds (Herbst, 2006). Under normal conditions, VEGF preferentially dilates arterioles and venules, and intravenous infusion of recombinant VEGF elicits reductions in blood pressure in humans through a mechanism thought to involve VEGF stimulation of protein kinase B, phosphorylation and increased activity of endothelial nitric-oxide synthase, subsequent enhanced NO production, and reduction in vascular tone (Lin and Sessa, 2006). Therefore, it is not unexpected that blockade of this pathway would result in hypertension, as recently demonstrated in patients treated with bevacizumab (Dincer and Altundag, 2006). It is noteworthy that 28% of patients treated with bevacizumab presented with grade 3 hypertension (greater than 180 mm Hg systolic pressure) (Shah et al., 2006). This effect is not limited to biologics targeting VEGF but also small molecules. In phase 1 clinical trials, ABT-869 decreases vascular permeability, as assessed by dynamic contrast-enhanced-magnetic resonance imaging in patients with refractory solid malignancies but also elicited reversible hypertension and proteinuria (Goh et al., 2006), an effect again presumably mediated via modulation of the VEGF-protein kinase B-endothelial nitric-oxide synthase pathway. However, it is not clear what class of antihypertensive agents will offer the optimal approach for controlling hypertension elicited by this agent or whether pharmacological approaches to control hypertension will affect the antitumor efficacy of RTK inhibitors.

Using a conscious telemetry-instrumented rat model, we systematically evaluated the effect of orally administered ABT-869 on blood pressure and subsequently characterized the dose-dependent effects of seven clinically efficacious antihypertensive agents encompassing three mechanistic classes (angiotensin-converting enzyme inhibitors (ACEis), angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs)) on hypertension induced by ABT-869. These three mechanistic classes of agent were chosen based on the clinical need for a rapid, potentially high amplitude reduction in arterial pressure. Finally, different agents from a class were tested because the known physicochemical properties of a given agent (i.e., distribution, tissue penetration) may determine the ultimate efficacy at reversing the hypertension. We also evaluated, in SCID-beige mice, whether controlling hypertension would attenuate or augment the antitumor efficacy of ABT-869. These results demonstrate that: 1) hypertension can be controlled by all three classes of antihypertensive agents, and the reductions in blood pressure showed a general rank order of ACEi > ARB > CCB; 2) that antihypertensive support can be initiated either at the onset of ABT-869 treatment or after hypertension is observed; and 3) that controlling hypertension elicits no detrimental effect on the ability of ABT-869 to suppress tumor growth.

### Materials and Methods

**Conscious Rat Blood Pressure Studies.** Male, Sprague-Dawley rats, 250 to 275 g, were anesthetized with Sevoflurane (Abbott Laboratories, Abbott Park, IL). Rats were placed on a heating pad and covered with a sterile surgical drape. A ventral midline abdominal incision was made, and sterile cotton tip applicators were used to gently move internal tissue and expose the abdominal aorta or implantation of the telemetry catheter (TA11PA-C40; Data Sciences International, St. Paul, MN). Blood flow was temporarily stopped to the lower extremities (5–7 min) with Diffenbach clamps to allow the insertion of the telemetry catheter into the abdominal aorta. Once inserted, a sterile cellulose patch was placed over the catheter/aorta and secured using a small amount of tissue adhesive (Vetbond; 3M, St. Paul, MN). Once catheter placement was complete, the clamps were removed, and blood flow was restored to the lower extremities. The transmitter was placed in the intraperitoneal cavity. The transmitter suture rib was sewn into the abdominal sutures to secure it in place. The skin was closed using sterile wound clips, and the animal was removed from Sevoflurane. Buprenex (0.01 mg/ml s.c.; Phoenix Pharmaceuticals, Belmont, CA) was administered for postoperative analgesia. Animals were maintained on a heating pad until ambulatory and then individually housed with food and water ad lib. Surgical staples were removed after 7 to 10 days of postimplantation. Rats were allowed a 2-week postsurgical recovery period before treatment with the test compound.

Instrumented rats were randomly divided into study groups and administered either vehicle or ABT-869 by oral gavage between 8:00 and 9:00 AM on days of treatment during each study; doses of drugs and variations (vehicle used was 0.2% hydroxypropyl methylcellulose) on the standard protocol unique to each study are described under Results. Entry into the telemetry room was kept minimal. All staff was instructed to log his/her time in and out of the room. The data collected during the time the staff was logged in were not included in the analysis. The telemetry catheter measured systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), and heart rate and were computed using commercial software and a signal processing workstation (Data Exchange Matrix; Data Sciences International); all parameters were continuously recorded at 15-min intervals for the duration of each study, and results are reported as the 24-h mean ± S.E.M. Pulse pressure was calculated by subtracting diastolic pressure values from systolic pressure values. Results are reported as 24-h mean ± S.E.; area under the curve (AUC) was calculated as millimeters of
Results

Conscious Rat Blood Pressure Studies

Dose-Dependent Increases in Blood Pressure Elicited by ABT-869. To elucidate the dose-dependent effects of ABT-869 blood pressure, ABT-869 was administered at 1.0, 3.0, 10.0, and 30.0 mg/kg p.o. q.i.d. for 5 days. Each study encompassed three phases: baseline (5 days), treatment period (5 days), and washout (5 days). During the treatment phase, the vehicle elicited no effect on SAP, DAP, MAP, or pulse pressure versus baseline (Fig. 2; SAP AUC = −6.13 ± 4.53, DAP AUC = −1.38 ± 3.01, MAP AUC = −3.71 ± 3.80, and pulse pressure AUC = −4.74 ± 1.70 mm Hg/day). The lowest dose of ABT-869 (1.0 mg/kg) produced no increase in systolic, diastolic, or mean arterial pressure AUC values (SAP AUC = −6.89 ± 4.25, DAP AUC = −3.44 ± 3.24, and MAP AUC = −5.52 ± 3.75 mm Hg/day) relative to baseline. However, the 3.0 mg/kg dose of ABT-869 produced significant increases in systolic, diastolic, and mean arterial pressure AUC values (SAP AUC = 8.53 ± 7.84, DAP AUC = 16.37 ± 9.12, and MAP AUC = 12.68 ± 8.38 mm Hg/day, respectively) versus vehicle-treated controls. Increases in SAP, DAP, and MAP AUC values were exacerbated at higher doses of ABT-869 (10.0 mg/kg; SAP AUC = 37.62 ± 5.03, DAP AUC = 36.48 ± 3.80, and MAP AUC = 37.47 ± 4.39 mm Hg/day; 30 mg/kg; SAP AUC = 61.56 ± 2.95, DAP AUC = 64.86 ± 4.13, and MAP AUC = 63.79 ± 3.26 mm Hg/day) versus vehicle-treated controls. During the treatment period, heart rate and pulse pressure AUC values were not changed, relative to vehicle controls, in any group administered ABT-869 (Fig. 2, A and B). Blood pressure values returned toward vehicle during posttreatment period in all groups administered ABT-869. Because systolic and diastolic arterial pressure AUC values were similar to mean arterial pressure AUC values, only mean arterial pressure values are shown in subsequent figures; see the supplemental data for corresponding heart rate values for each group. Plasma concentrations of ABT-869 (10.0 mg/kg) at 1.5 and 24 h postdose are shown in Fig. 2C; peak concentrations of ABT-869 on each day of treatment ranged between 90 and 164 ng/ml, and the nadir concentration reached between 5 and 31 ng/ml.

Effect of ACE Inhibitors (Enalapril, Lisinopril, Ramipril) to Prevent Hypertension Elicited by ABT-869. To determine whether increases in blood pressure elicited by ABT-869 could be prevented by ACE inhibitors, ABT-869 (10.0 mg/kg p.o.) was administered q.i.d. concomitant with oral administration of enalapril, lisinopril, or ramipril for 5 days as described below; each study encompassed three phases: baseline (5 days), treatment period (5 days), and washout (5 days).

Enalapril (0.3, 1.0, 3.0, 10.0, or 30.0 mg/kg) was administered orally q.i.d., concomitant with oral administration of enalapril, lisinopril, or ramipril for 5 days as described below; each study encompassed three phases: baseline (5 days), treatment period (5 days), and washout (5 days).
−32.98 ± 3.12 mm Hg/day, respectively) versus ABT-869 alone; values were decreased below that of vehicle-treated controls at 10.0 and 30.0 mg/kg. During the treatment period, heart rate AUC values were not changed, relative to vehicle controls, in any group administered enalapril. However, heart rate AUC values trended down relative to vehicle-treated controls and enalapril at 0.3, 1.0, and 30.0 mg/kg significantly increased versus ABT-869 alone (Supplemental Fig. 1A).

Lisinopril (0.3, 1.0, 3.0, or 10.0 mg/kg) was administered orally q.i.d., concomitant with ABT-869 (10 mg/kg p.o. q.i.d.) (Supplemental Table 1). During the treatment period, vehicle elicited no effect on blood pressure versus baseline (Fig. 3B; MAP AUC = 8.61 ± 2.02 mm Hg/day). ABT-869 produced an increase in mean arterial pressure, to an average of 13 mm Hg above baseline (range = 8–11 mm Hg) during 5 days of treatment (MAP AUC = 40.47 ± 3.38 mm Hg/day). All doses of lisinopril (0.3, 1.0, 3.0, and 10.0 mg/kg) significantly decreased MAP AUC (20.08 ± 3.19, 2.6 ± 4.33, 9.56 ± 7.23, and −36.06 ± 4.89 mm Hg/day) relative to ABT-869; the two highest doses (3.0 and 10.0 mg/kg). MAP AUC values were decreased versus vehicle-treated controls. During the treatment period, heart rate AUC values were not changed, relative to vehicle controls or ABT-869, in any group administered lisinopril (Supplemental Fig. 1B).

Ramipril (0.1, 0.3, 1.0, and 3.0 mg/kg) was administered orally q.i.d., concomitant with ABT-869 (10 mg/kg p.o. q.i.d.) (Supplemental Table 1). During the treatment period, vehicle elicited no effect on blood pressure versus baseline (Fig. 3C; MAP AUC = 13.60 ± 1.96 mm Hg/day). ABT-869 produced an increase in mean arterial pressure to an average of 13 mm Hg above baseline (range = 11–15 mm Hg) during 5 days of treatment (MAP AUC = 54.71 ± 2.99 mm Hg/day). The lowest dose of ramipril (0.1 mg/kg) produced no effect on MAP AUC (47.36 ± 5.77 mm Hg/day) versus ABT-869 alone. However, the three highest doses of ramipril (0.3, 1.0, and 3.0 mg/kg) dose-dependently and significantly decreased MAP AUC (30.53 ± 4.33, 10.45 ± 5.92, and 2.23 ± 8.45 mm Hg/day, respectively) relative to ABT-869 alone to values not different from vehicle-treated controls. During the treatment period, heart rate AUC values were not changed, relative to vehicle controls or ABT-869, in any group administered ramipril (Supplemental Fig. 1C).

**Effect of Angiotensin Receptor Blockers (Telmisartan, Eprosartan) to Prevent Hypertension Elicited by ABT-869.** To determine whether increases in blood pressure
Eprosartan (10.0, 30.0, or 60.0 mg/kg) was administered orally q.i.d., concomitant with ABT-869 (10 mg/kg p.o. q.i.d.) (Supplemental Table 1). During the treatment period, vehicle elicited no effect on blood pressure versus baseline (Fig. 4B; MAP AUC = 13.60 ± 1.96 mm Hg/day). ABT-869 produced an increase in mean arterial pressure to an average of 13 mm Hg above baseline (range = 10–16 mm Hg) during 5 days of treatment (MAP AUC = 54.71 ± 2.99 mm Hg/day). The low dose of eprosartan (10.0 mg/kg) produced no effect on MAP AUC values (41.12 ± 3.16 mm Hg/day) relative to ABT-869. However, the two highest doses of eprosartan (30.0 and 60.0 mg/kg) significantly decreased MAP AUC (18.48 ± 11.02 and 27.74 ± 8.36 mm Hg/day, respectively) relative to ABT-869 alone. During the treatment period, heart rate AUC values were not changed, relative to vehicle controls or ABT-869, in any group administered eprosartan (Supplemental Fig. 2B).

Effect of Calcium Channel Blockers (Nifedipine, Amiodipine) to Prevent Hypertension Elicited by ABT-869. To determine whether increases in blood pressure elicited by ABT-869 could be prevented by calcium channel blockers, ABT-869 (10.0 mg/kg p.o.) was administered q.i.d. concomitant with oral administration of nifedipine or amlo-


duced by ABT-869 could be prevented by angiotensin receptor blockers, ABT-869 (10.0 mg/kg p.o.) was administered q.i.d. concomitant with oral administration of telmisartan or eprosartan for 5 days as described below; each study encompassed three phases: baseline (5 days), treatment period (5 days), and washout (5 days).

Telmisartan (1.0, 3.0, or 10.0 mg/kg) was administered orally q.i.d., concomitant with ABT-869 (10 mg/kg p.o. q.i.d.) (Supplemental Table 1). During the treatment period, vehicle elicited no effect on blood pressure versus baseline (Fig. 4A; MAP AUC = −13.64 ± 3.83 mm Hg/day). ABT-869 produced an increase in mean arterial pressure to an average of 12 mm Hg (range = 8–13 mm Hg) above baseline during 5 days of treatment (MAP AUC = 48.20 ± 2.84 mm Hg/day). All doses of telmisartan (1.0, 3.0, and 10.0 mg/kg) significantly decreased MAP AUC (16.88 ± 7.89, 8.83 ± 5.20, and −3.14 ± 6.47 mm Hg/day, respectively) relative to ABT-869. However, MAP AUC values at 1.0 and 3.0 mg/kg remained elevated versus vehicle-treated controls. During the treatment period, heart rate AUC values were not changed, relative to vehicle controls or ABT-869, in any group administered telmisartan (Supplemental Fig. 2A).
Nifedipine (3.0, 10.0, or 30.0 mg/kg) was administered orally q.i.d., concomitant with ABT-869 (10 mg/kg p.o. q.i.d.) (Supplemental Table 1). During the treatment period, vehicle elicited no effect on blood pressure versus baseline (Fig. 5A; MAP AUC = 4.39 ± 1.98 mmHg/day). ABT-869 produced an increase in mean arterial pressure to an average of 12 mm Hg (range = 9–14 mm Hg) above baseline during 5 days of treatment (MAP AUC = 49.49 ± 2.83 mmHg/day). The lowest dose of nifedipine (3.0 mg/kg) produced no effect on MAP AUC (39.68 ± 3.25 mm Hg/day) versus ABT-869 alone. The two highest doses of nifedipine (10.0 and 30.0 mg/kg) significantly decreased MAP AUC (23.03 ± 4.26 and 13.70 ± 3.09 mm Hg/day, respectively) relative to ABT-869, however, at 10.0 mg/kg values remained elevated versus vehicle-treated controls. During the treatment period, heart rate AUC values...
were not changed, relative to vehicle controls or ABT-869, in any group administered nifedipine (Supplemental Fig. 3A).

Amlodipine (0.3, 1.0, 3.0, or 10.0 mg/kg) was administered orally q.i.d., concomitant with ABT-869 (10 mg/kg p.o. q.i.d.) (Supplemental Table 1). During the treatment period, vehicle elicited no effect on blood pressure versus baseline (Fig. 5B; MAP AUC = 6.73 ± 1.34 mm Hg/day). ABT-869 produced an increase in mean arterial pressure to an average of 16 mm Hg (range = 11–19 mm Hg) above baseline during 5 days of treatment (MAP AUC = 65.60 ± 4.04 mm Hg/day). The lowest doses of amlodipine (0.3, 0.1, and 3.0 mg/kg) significantly decreased MAP AUC (47.09 ± 1.86, 45.90 ± 2.76, and 37.56 ± 4.44 mm Hg/day, respectively) versus ABT-869 alone, however, remained elevated versus vehicle-treated controls. The highest dose of amlodipine (10.0 mg/kg) significantly decreased MAP AUC values (2.86 ± 3.43 mm Hg/day) versus ABT-869 alone to values not different from vehicle controls. During the treatment period, mean heart rate AUC values were significantly increased relative to ABT-869 and vehicle-treated controls only in the group that received amlodipine at 10 mg/kg (Supplemental Fig. 3B).

**Effect of the ACE inhibitor, Lisinopril, to Reverse Sustained Hypertension Elicited by ABT-869.** To determine whether increases in blood pressure elicited by ABT-869 for 5 days could be reversed by lisinopril at doses shown to be effective in the prevention study, ABT-869 (10.0 mg/kg p.o.) was administered q.i.d. concomitant with oral administration of lisinopril for 5 days as described below; the study encompassed five phases: baseline (5 days), ABT-869 alone (5 days), ABT-869 plus lisinopril (7-days), ABT-869 alone (5 days), and washout (5 days).

Lisinopril (0.3, 1.0, or 3.0 mg/kg) was administered orally q.i.d., concomitant with ABT-869 for 7 consecutive days before washout, followed by another 5 consecutive days of ABT-869 alone. Vehicle elicited no effect on blood pressure versus baseline throughout days 1 to 12 of the study [Fig. 6, A and B; days 1–5 (ABT-869 alone) MAP AUC = 11.81 ± 3.3 + 2 mm Hg/day; days 6–12 (combination treatment) MAP AUC = 24.84 ± 5.38 mm Hg/day]. ABT-869 alone produced a sustained increase in mean arterial pressure to an average of 12 mm Hg (range = 9–15 mm Hg) above baseline over 17 days of treatment (days 1–5 (ABT-869 alone) MAP AUC = 54.04 ± 3.84 mm Hg/day; days 6–12 (combination treatment) MAP AUC = 73.59 ± 4.89 mm Hg/day). All three doses of lisinopril (0.3, 1.0, or 3.0 mg/kg) dose-dependently and significantly reversed increases in MAP AUC (33.35 ± 8.61, 10.86 ± 3.84, and −9.18 ± 7.37 mm Hg/day, respectively) relative to ABT-869 during combination treatment. In the presence of the highest dose of lisinopril (3.0 mg/kg), MAP AUC fell to values below that of vehicle-treated controls. Upon withdrawal of lisinopril, MAP AUC values at all three doses of lisinopril returned to values similar to that of ABT-869 alone (30.06 ± 5.08, 27.18 ± 2.75, and 21.81 ± 6.27 mm Hg/day, respectively). MAP decreased toward baseline and was similar to that of vehicle controls during the 5-day washout phase. Heart rate AUC values were not changed, relative to vehicle-treated controls or ABT-869, in any group administered lisinopril for the duration of the lisinopril treatment period (Supplemental Fig. 4, B and C).

**Blood Pressure Studies in Conscious SCID-Beige Mice.** To assess any potential effects of antihypertension therapy on the antitumor activity of ABT-869, blood pressure, and tumor growth studies were conducted in SCID-beige mice. After instrumentation, vehicle produced no effect on mean arterial pressure throughout 14 days of treatment. Administration of ABT-869 (12.5 or 25 mg/kg/day p.o. b.i.d.), doses sufficient to produce tumor growth inhibition, produced immediate and sustained hypertension (18–20 mm Hg above baseline) throughout the 14-day treatment period. Concomitant administration of enalapril (30.0 mg/kg p.o. q.i.d.) completely prevented the hypertensive effect of both doses of ABT-869 (Fig. 7A). During the post-treatment pe-
period, mean arterial pressure returned to baseline values and were not different from vehicle controls (Fig. 7A). No treatment protocol elicited any physiologically relevant effects on heart rate throughout the study (Supplemental Fig. 5).

Inoculation of SCID-beige mice with HT-1080 cells produced tumors between 0.35 and 0.50 ml on day 7 (Fig. 7B) that increased to approximately 3.5 ml by day 21. Administration of ABT-869 (12.5 mg/kg/day, days 7–21) completely suppressed tumor growth. Co-administration of enalapril at a dose sufficient to prevent ABT-869-induced hypertension (30 mg/kg/day) had no effect on the antitumor efficacy of ABT-869. Enalapril given as monotherapy had no significant effect on tumor growth.

**Discussion**

VEGF-targeted chemotherapeutic agents are of substantial benefit to cancer patients (Faiivre et al., 2006) but elicit reversible hypertension that represents a class effect for these compounds that presumably is due to the effect of VEGF on vascular tone. Consistent with these observations, the present study demonstrates that: 1) ABT-869 elicits dosedependent increases in mean arterial pressure in conscious, telemetry-instrumented freely moving rats; and 2) increases in blood pressure elicited by ABT-869 can be effectively controlled with ACEIs, ARBs, and CCBs, and the reduction in blood pressure shows a general rank order of ACEi > ARB > CCB. Moreover, at doses of lisinopril (ACEi) shown to prevent hypertension in the present study, we also demonstrate that the compound can effectively reverse sustained increases in blood pressure produced by prior treatment with ABT-869. The hemodynamic response to ABT-869 has not been defined in animals with pre-existing hypertension. Finally, we demonstrate that hypertension elicited by ABT-869, in SCID-beige mice, can be completely reversed by enalapril, with no attenuation of the antitumor efficacy of the molecule.

In the present study, there are qualitative differences in the ability of agents within and between mechanistic classes to lower blood pressure after ABT-869 [ACEi (largest reduction in blood pressure) lisinopril > ramipril ≥ enalapril (smallest reduction in blood pressure); ARB, eprosartan > telmisartan; CCB, nifedipine > amlodipine (see Fig. 8)]. We also demonstrate that lisinopril can effectively control hypertension elicited by ABT-869 when administered either at the onset of therapy or after sustained hypertension is already present. In fact, at doses even below that normally administered to patients to control hypertension (normal dosing range = 20.0–40.0 mg/day), lisinopril effectively prevented and reversed hypertension subsequent to ABT-869 treatment. These preclinical studies suggest that in clinical practice, antihypertensive therapy does not necessarily need to commence concomitant with initial ABT-869 therapy but rather can be managed when observed in patients. Additional hypertension reversal studies with the ACE inhibitor enalapril (30.0 mg/kg/day) and the CCB amlodipine (10.0 mg/kg/day) demonstrated that both compounds fully reduced blood pressure similar to lisinopril, suggesting that this effect is not exclusive to lisinopril or solely due to a mechanism involving modulation of the renin angiotensin system (data not shown). Although the present study did not examine the utility of diuretics and/or β-adrenergic blocking agents, these agents are employed less frequently clinically because of VEGF blockade, causing a rapid onset and (in certain patients) high-amplitude increases in blood pressure.

Results from studies in conscious telemetry-instrumented SCID-beige mice clearly demonstrate that reversal of hypertension, in this case with an ACE inhibitor, does not affect the antitumor efficacy of ABT-869. In fact, tumor stasis in the presence of ABT-869 was indistinguishable from that of
ABT-869 in the presence of enalapril and was independent of any effects of enalapril alone on tumor growth. Previous studies have provided evidence that ACE inhibitor can either suppress tumor growth and angiogenesis in experimental animal models (Rivera et al., 2001; Yoshiji et al., 2001; Fujita et al., 2002; Miyajima et al., 2002; Egami et al., 2003; Arrieta et al., 2005; Arafat et al., 2007) or stimulate angiogenesis and reverse microvascular vascular rarefaction associated with hypertension, which could lead to increased tumor growth (for review, see Battegay et al., 2007). Neither of these outcomes was evident in the current study.

While this work was in progress, an opinion article by Dincer and Altundag (2006) suggested that ACE inhibitors should be considered as the antihypertensive medication of choice to control hypertension produced by bevacizumab. In contrast, a recent report of studies with another VEGF signaling inhibitor indicated that nifedipine was more effective than the ACE inhibitor captopril in controlling hypertension elicited by cediranib (Curwen et al., 2008). Results from the present study, in conscious telemetry-instrumented rats, are consistent with the former recommendation and further advance the field by demonstrating modest differences between that of an ACE inhibitor and an ARB or CCB to control hypertension elicited by ABT-869. Furthermore, we demonstrate that hypertension can be both prevented and reversed at therapeutic or even subtherapeutic doses and that control of hypertension elicits no attenuation of the antitumor efficacy of the molecule.

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References

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