ZD6126: A Novel Vascular-targeting Agent That Causes Selective Destruction of Tumor Vasculature


INTRODUCTION

A functioning vascular network is crucial for the survival and growth of solid tumors (1), making tumor blood vessels a key target for drug discovery in oncology. Most of the work in this area has focused on the development of antiangiogenic approaches to cancer therapy, and several agents are undergoing clinical evaluation. Examples include peptide inhibitors of endothelial cell proliferation, angiostatin (2) and endothastin (3), and inhibitors of the tyrosine kinase activity of flk-1/KDR, a receptor for vascular endothelial growth factor (4). An alternative to this antiangiogenic approach is to target the existing tumor vasculature (5). Differences in the physiology of immature tumor versus mature normal vasculature provide an opportunity for the selective disruption of tumor blood flow, leading to tumor death. A potential advantage of this approach is the targeting of central areas of a tumor that can be resistant to conventional therapy.

Certain tubulin-binding agents have been shown to induce vascular damage in mouse tumor models, but only at, or approaching, the MTD (6, 7). More recently, another tubulin-binding agent, combretastatin A-4 phosphate was demonstrated to have this activity at doses of one-tenth of MTD (8). The effects of tubulin-binding agents on tumor vasculature has been ascribed to a direct effect on endothelial cells (9).

The present study describes the synthesis and characterization of a novel vascular-targeting agent. The agents that have been previously shown to have vascular-targeting activity only at MTD are either slowly reversible tubulin-binding agents or have extended elimination half-lives in vivo. Our hypothesis was that successful vascular-targeting agents would have reversible binding kinetics and relatively rapid clearance in vivo. The direct effects on endothelial cells postulated in the mechanism of action of tubulin-binding agents occur over a short time period, and vascular damage in vivo is evident within minutes (10). Therefore, short exposures should be sufficient to elicit effects on tumor vasculature but may be less effective in generating the common toxicities associated with tubulin-binding agents.

NAC is structurally removed from colchicines in that it contains a benzenoid ring in place of the tropolone ring in colchicine. Unlike colchicines, members of the colchinol family have been shown to bind to tubulin with rapidly reversible kinetics (11). Also, NAC bears a phenolic hydroxy group that we thought may result in rapid elimination (probably through glucuronidation) in common with many phenolic compounds. Finally, the phenol group may facilitate synthesis of a water-soluble prodrg.

In this article, we report the synthesis of ZD6126 and show the induction of pronounced but reversible changes in immature endothelial cell morphology at subcytotoxic doses. In an experimental tumor model, ZD6126, at doses well below the MTD, is shown to have pronounced vascular effects and antitumor activity that is consistent with an antivascular rather than a cytotoxic mechanism.

MATERIALS AND METHODS

Synthesis. Fig. 1 illustrates the synthesis of (5S)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,e]cyclohepten-3-yl dihydrogenphosphate (ZD6126) from colchicines. Steps A and B were performed according to the published procedure (12). Step C was performed as follows: NAC (7.5 g, 21.9 mmol) was dissolved in anhydrous tetrahydrofuran (60 ml) and stirred, under nitrogen, at room temperature together with di-tert-butyldiethylphosphoramidite (6.54 g, 26.3 mmol) and 1H-tetrazole (4.0 g, 57.4 mmol). After 2 h, the solution was cooled to ~40°C, and a solution of 3-chloroperoxybenzoic acid (5.82 g, 28.6 mmol) in dichloromethane (60 ml) was added such that the temperature remained below ~20°C (monitored with thermocouple). The solution was warmed to 0°C to 4°C and a saturated solution of sodium bicarbonate (100 ml) was added, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with saturated sodium bicarbonate solution and dried over MgSO4. Solvents were removed in vacuo to give 7.0 g of a light yellow oil. Purification on silica gel (dichloromethane/methanol, 98:2) afforded 8.24 g (71%) of N-acetylcolinol-di-tert-buty phosphate as a white waxy solid. A portion of this solid (6.5 g, 11.0 mmol) was dissolved in dichloromethane (150 ml) and trifluoroacetic acid (19 mmol) was added. After 1 h, the solution was filtered and evaporated to dryness. The residue was dissolved in dichloromethane (30 ml) and stirred at room temperature for 3 h before being filtered and evaporated to give an oil; the mixture was dried over MgSO4 overnight. Removal of solvents afforded 3.068 g (70%) of ZD6126. The crude product was treated with a mixture of tetrahydrofuran/methanol (40:1) and stirred for 2 h, before being filtered and evaporated to give a residue, which was washed with petroleum ether (b.p. 60–80°C) before being dried under vacuum to give ZD6126 (2.89 g, 70% yield; 2.2 g, 69% yield) without further purification.

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2 The abbreviations used are: NAC, N-acetylcolinol; MTD, maximum tolerated dose; HUVEC, human umbilical vein endothelial cell.
ml, 247 mmol) added slowly with cooling and stirring in an ice/water bath. The temperature was allowed to rise to a maximum of 1.5 h. Solvent and trifluoroacetic acid were removed in vacuo at 30° C and the product was triturated with diethyl ether (100 ml), filtered, and washed well with diethyl ether. The white solid thus obtained was recrystallized from ethanol to give 3.8 g (73%) of ZD6126 as a white, finely crystalline solid of melting point 248° C to 249° C.

For in vivo experiments, ZD6126 was dissolved in water, and 5% aqueous sodium carbonate solution was added dropwise until a clear solution was obtained.

Tubulin Binding Properties. Tubulin binding properties were evaluated using a colchicine-binding-site competition assay and a commercially available kit (Cytoskeleton Inc, Denver, CO). Test compounds at various concentrations were incubated for 30 min at 37° C with 1 mg/ml purified bovine brain tubulin. Controls comprised medium alone and an excess of colchicine (100 μM) giving maximum competition of fluorescent colchicine binding to tubulin. Fluorescently labeled colchicine (0.5 μM) was then added to the test-plus-tubulin mixture and incubated for an additional 30 min at 37° C. After incubation, the sample was transferred to a G25 Sephadex column, and the eluent that contained colchicine-bound tubulin was collected in a 96-well plate and read spectrophotometrically with excitation at 485 ± 20 and emission at 530 ± 20 nm.

Cytotoxicity in Vitro. HUVECs that had been isolated as primary explants were dosed with compound for 20 h, washed twice in PBS, and 10% FCS was added for 15 min. For the recovery experiments, incubation of proliferating cells with the drug was performed as described above; then the coverslips were washed four times with serum-free medium, and medium was replaced for 24 h before fixation as above.

Fresh 6-cm Petri dishes were used for staining. Primary mouse monoclonal anti-β tubulin antibody (120 μl; Sigma) was placed in each dish at 1-in-500 or 1-in-1000 dilution for confluent or proliferating cells, respectively. The coverslips were then carefully inverted onto the antibody solution, and incubated at 37° C for 1 h. Two ml of 10% FCS were added to each dish, and the coverslips were carefully reinverted and replaced in their original dishes. After two additional washes in 10% FCS, the staining procedure was repeated for the secondary antibody, FITC-linked antimouse antibody (Sigma) at 1-in-25 and 1-in-50 dilution for confluent and proliferating cells, respectively; and the medium was incubated for 30 min. The coverslips were then reinverted into their original dishes, washed twice in PBS and mounted onto glass slides using anti-fade fluorescent mounting medium. Experiments were repeated three times with HUVECs from different umbilical cords.

The tubulin-stained cells were visualized under blue excitation light, and photographs were taken using an Olympus BH2 fluorescence microscope and Kodak ASA 200 film. For quantitation of cell shape, images were acquired and stored on computer using a Nikon Optiphoto fluorescence microscope, camera, and in-house image acquisition software. Images were acquired from randomly selected areas of slides and then were analyzed using Visilog v4 software (Noesis, Paris, France), adapted in-house. On each image, a light pen was used to draw around each cell outline, using the zoom feature to increase magnification to ×850. The perimeter and cell area (in pixels) contained by the outline were calculated. One pixel measured 0.71 ± 0.71 μm. At least 80 cells were outlined in this way for each slide. Cells completely imaged at the edge of the field were excluded.

For dual staining of tubulin and actin, cells were plated, treated (for 2 h), and fixed as described above. Texas Red-phalloidin (Molecular Probes Europe BV) was obtained as lyophilized solids in a 300-unit vial. Stock solution was made by dissolving the solids in 1.5 ml of methanol to yield a final concentration of 200 units/ml. The normal tubulin-staining procedure was followed but at the secondary-antibody stage, 5 μl of solution per slide to be stained was added to the secondary-antibody solution and mixed. One hundred twenty-five μl of the combined solution was place on each slide and incubated as above. Dual color photographs were obtained by exposure, using blue excitation light to obtain an image of tubulin, followed by a second exposure under green light to obtain an image of actin. The microscope stage was not moved between exposures. Dual-staining data shown are representative of the areas examined.

Pharmacokinetics and Protein Binding. CBA mice were dosed i.p. with 200 mg/kg ZD6126 at various times before sacrifice and exanguination. Blood samples were collected into EDTA (final concentration, 2 mg/ml) and centrifuged at 12,000 × g for 1 min; separated plasma was stored at −20° C. Aliquots (50 μl) were treated with methanol (1 ml) and an internal standard, centrifuged (12,000 × g) for 2 min, and the supernatants dried in a centrifugal evaporator. Samples were reconstituted in 40% aqueous acetonitrile analyzed for both ZD6126 and NAC by high-performance liquid chromatography on a 250 × 5.2-mm Hichrom RPb column with a gradient starting with a mixture of 45% methanol, 55% 1-butanol, 0.01% trifluoroacetic acid, and 0.55 mM potassium dihydrogen phosphate, to 45% methanol, 30% acetonitrile, 25% water. Detection was performed at Wates 996 diode array at 260 nm.

Protein binding was evaluated by ultrafiltration (1500 × g, 20 min) of plasma samples spiked with radiolabeled NAC at five different concentrations (0.01, 0.1, 1.0, 3.0, and 10.0 μg/ml) followed by determination of radioactivity in the ultrafiltrate.

In Vivo Studies. All animal procedures were carried out under a project license issued by the Home Office, London, United Kingdom and were performed according to United Kingdom guidelines (14). Mice were given access to food and water ad libitum. Experiments were performed using either the syngeneic breast adenocarcinoma, CaNT, grown in CBA mice or the
human pharynx squamous cell carcinoma, FaDu, grown in severe-combined immunodeficient mice. Tumors were used at a starting geometric mean diameter of between 5 and 6 mm. To guide in vivo studies, experiments were carried out to determine the MTD of ZD6126 after i.p. administration. The studies adhered to guidelines published previously (15). Because of ethical considerations, the MTD was not accurately determined but was approximated at 750 mg/kg, a dose that resulted in no deaths in three female mice. In contrast, the MTD of NAC approximated 125 mg/kg.

Functional vascular volume was assessed 6 h after drug treatment following a published procedure (8). CaNT tumor-bearing mice were treated i.p. with ZD6126 at 25, 50, and 125 mg/kg. Six h later, the DNA-binding fluorescent dye Hoechst 33342 (10 mg/kg) was injected via the tail vein into treated and untreated mice. One min later, the mice were sacrificed and tumors excised, frozen, sectioned, and visualized under UV excitation. Hoechst 33342 can be visualized using UV excitation at 376 nm, with a 418-nm-long pass barrier filter. Blood vessels are identified by their fluorescent outlines and vascular volume is quantified using a point-scoring system. A grid of 25 points is focused on an area of the section, and points falling inside rings of fluorescent cells are scored as positive. This is repeated, moving randomly over different areas of the tissue, until 3000 points in total have been accumulated from sections cut at the three different levels (i.e., by scoring 120 fields/tumor level). This sample size was chosen to give a relative SE of ~10% on the measured volume fraction. The vascular volume fraction for each sample is calculated as the ratio of positive:total points. Results were expressed as percentage vascular volume compared with control tumors.

Histological assessment was made to assess the induction of necrosis. CaNT-bearing mice were treated i.p. with ZD6126, and tumors were excised 24 h later. After fixation in formalin, sections were made from paraffin-embedded tumors and stained with H&E. Sections were scored by eye according to the following scale: 1, 0–10% necrosis; 2, 11–20% necrosis; and so on until 10, 91–100% necrosis.

The level of tumor cell kill by ZD6126 was measured in the CaNT model by clonogenic assay of tumors excised 18 h after i.p. drug administration as described previously (16). Tumors were weighed and disaggregated for 30 min at 37°C in an enzyme cocktail containing 1 mg/ml pronase, 0.5 mg/ml DNAse and 0.5 mg/ml collagenase. Hemocytometer counts of trypan blue-excluding cells were made and viable cells seeded in appropriate concentrations to yield about 0.5 mg/ml collagenase. Hemocytometer counts of trypan blue-excluding cells were made and viable cells seeded in appropriate concentrations to yield about 50 colonies/dish after in vitro incubation. Heavily irradiated feeder cells (V79 cells) were used at a concentration of 25,000/ml to support the growth of the surviving CaNT cells. The data were calculated as relative surviving fraction per gram of tumor, relative cell yield per gram of tumor, and surviving fraction per gram of tumor, the latter being the product of surviving fraction and cell yield.

Tumor growth was measured after the i.p. dose administration. Tumor dimensions were measured in three orthogonal diameters using calipers. Five mice were included per treatment group. Growth delay was determined by the difference in time taken to grow 3 mm (geometric mean diameter) larger than the original size at treatment minus the time for controls to do the same. Statistical comparisons were performed using Student’s t test.

RESULTS

Tubulin Binding Properties. ZD6126 and NAC were evaluated for their ability to compete with fluorescently labeled colchicine binding to purified bovine tubulin. ZD6126 was inactive in the assay at concentrations up to 100 μM. NAC inhibited 50% of the fluorescently labeled colchicine binding at 5.4 μM (mean of three studies), whereas unlabelled colchicine inhibited 50% of the binding at 4.5 μM (mean of two studies). These studies demonstrated that, whereas NAC was a potent tubulin binding agent, the prodrug ZD6126 had minimal activity.

Cytotoxicity in Vitro. Cytotoxicity of ZD6126 and NAC toward HUVEC monolayers (Fig. 2) was evaluated using a short exposure similar to that obtained in the in vitro experiments. The compounds had equivalent activity, which suggested rapid conversion of ZD6126 into NAC under the assay conditions. Neither compound at <30 μM showed a significant effect on HUVEC growth.

Effects on Endothelial Cell Morphology. A study was made of the morphological effects of ZD6126 on confluent and nonconfluent HUVEC monolayers to model the endothelial cells of mature and immature vasculature, respectively. Unlike endothelial cells of mature vasculature, those of immature vasculature, e.g., in areas of regenerating endothelium, have no well-developed actin filament structure (observed in mature cells as a “dense peripheral band”) supporting their shape (17). Because tumor vasculature bears the hallmarks of immaturity (18) and has areas of active angiogenesis, it is likely that endothelial cell morphology within this vascular bed resembles that in other immature, regenerating vasculature with regard to actin cytoskeletal organization. Confluent HUVEC monolayers exhibited the dense peripheral band of actin, whereas the actin microfilament distribution on the nonconfluent cells appeared diffuse (Fig. 3). Treatment of the culture with 0.1 μM ZD6126 resulted in changes to the antitubulin antibody staining pattern consistent with the degree of tubulin depolymerization in both confluent and nonconfluent monolayers, but a dramatic collapse of cell shape was apparent only in the nonconfluent culture. Although this is a crude model of mature and immature endothelium, it supports the hypothesis that differences in actin cytoskeletal organization may contribute to differential sensitivity to ZD6126.

The response of endothelial cell shape to increasing concentrations of NAC is shown in Fig. 4. Cell area reduction was apparent at 0.1 μM, maximal at 10 μM, and fully reversible at all but the highest concentration (100 μM). This lack of reversibility may be a reflection of cytotoxicity at the high concentration.

Pharmacokinetics and Protein Binding. The plasma concentration of ZD6126 and NAC was evaluated in female CBA mice after a single i.p. dose of 200 mg/kg ZD6126 (Fig. 5). Rapid prodrug activation followed by rapid elimination of NAC was apparent. The maximum concentration of NAC was ~200 μM. This would suggest, given linear pharmacokinetics, that the concentrations reached at the effective dose of 25 mg/kg would be in the region of 25 μM, below the concentration at which endothelial cell growth inhibition is seen in vitro but above that at which shape change is observed. The plasma level profile obtained, therefore, supports a mechanism of action in which the compound acts to induce changes in endothelium without being directly cytotoxic to endothelial cells. NAC was 84% protein bound in mouse plasma, which may reduce the concentration of free, active drug.

In Vivo Studies. ZD6126 reduced functional vascular volume in the CaNT mouse mammary adenocarcinoma in a dose-dependent manner (Fig. 6). A dose of 25 mg/kg, 30-fold lower than the MTD in these mice, was significantly active. At 24 h after drug administration,
ZD6126 caused massive necrosis in the central region of CaNT tumors, and only a small viable rim of viable cells remained at the higher doses (Fig. 7). Significant necrosis was seen at doses down to 25 mg/kg. Median necrosis scores obtained were as follows: control, 1.5 (n = 8); 25 mg/kg ZD6126, 5.5 (n = 8; significantly different from control by Mann-Whitney t test, P < 0.005); 125 mg/kg ZD6126, 9.0 (n = 5; significantly different from control by Mann-Whitney t test, P < 0.005). Early necrosis is characteristic of cell death by metabolic starvation (17–19).

Early cell death and clonogenicity of remaining cells was assessed quantitatively by an excision assay (Fig. 8). Because metabolic starvation can lead to rapid cell lysis and loss, we determined both the surviving fraction of the cells recovered and the relative changes in cell recovery or yield per gram of tumor, and calculated the product of these parameters to give a measure of the overall cell kill. Eighteen h after the administration of 200 mg/kg more than 95% of the tumor cells were killed and 80% of the killing was manifested by a reduced tumor cell yield. This finding is consistent with the widespread tumor necrosis seen histologically 24 h after drug administration and is again consistent with antivascular rather than cytotoxic effects. Although a dose of 50 mg/kg ZD6126 gave close-to-maximal vascular volume loss, significant increases in cell kill were seen above this dose. This most likely is a consequence of the fact that the vascular volume assay is a “snapshot,” taken at 6 h after dose administration, whereas the level of cell kill, measured in this case at 18 h, is a function of both the degree of oxygen/nutrient starvation and the length of time for which it persists. Recovery of vascular volume at 18 h after drug administration has been reported for combretastatin A-4 phosphate (8).

Despite the widespread necrosis and reduction in cell yield after ZD6126 administration, well-tolerated doses of ZD6126 produced only modest growth delay in CaNT tumors (Fig. 9A). The remaining viable cells in the tumor rim preserve the measured size of the tumor, despite the death of the inner region and may contribute to a rapid regrowth after single-dose treatment. Multiple well-tolerated doses, however, induced long growth delays (Fig. 9B, and the differences were statistically significant (P < 0.05). Tumor growth delays (± SE)
were 8.7 ± 0.9 days for the 2-week treatment and 14.2 ± 0.9 days for the 3-week treatment. Maximum weight loss in these groups of mice was 0.2 ± 2.5% for the 2-week treatment and 3.6 ± 1.8% for the 3-week treatment.

In the FaDu xenograft, the growth delay attributable to a single dose of paclitaxel was enhanced by combination with a single dose of ZD6126 (Fig. 10). Tumor growth delays (± SE) were 5.1 ± 0.3 and 3.9 ± 0.8 days, respectively, for paclitaxel at 15 and 30 mg/kg; 4.3 ± 1.3 days for ZD6126 at 125 mg/kg; and 14.1 ± 1.3 days for the combination with 15 mg/kg paclitaxel administered 15 min before 125 mg/kg ZD6126. Thus, the growth delay from the combination was greater than the sum of the growth delays from the individual treatments.

DISCUSSION

ZD6126 is an effective prodrug of the tubulin-destabilizing agent NAC. Although the prodrug showed minimal activity, the active drug was a potent inhibitor of colchicine binding. Despite the tubulin-binding activity of NAC, neither the prodrug nor the active product was cytotoxic toward endothelial cells under the conditions of drug exposure reflecting the exposure obtained in vivo.

ZD6126 has potent effects on tumor vasculature, evident within 24 h after dosing. Significant effects on tumor vascular volumes were seen at approximately one-thirtieth of the MTD, which compares with data for combretastatin A-4 phosphate, which shows effects on tumor vasculature at doses at approximately one-tenth of the MTD in the same experimental tumor model (8). Other agents that can elicit

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Fig. 7. Induction of necrosis by ZD6126 in CaNT tumors. Tumors were excised 24 h after i.p. administration of 125 mg/kg ZD6126. H&E staining: A, control (bar, 1 mm); B, treated (bar, 0.1 mm); C, control (bar, 0.1 mm); D, treated (bar, 0.1 mm).

Fig. 8. Tumor cell survival 18 h after i.p. ZD6126 administration. Tumors were excised and clonogenic assays performed. A, surviving fraction per gram of tumor; B, surviving fraction; C, relative yield per gram tumor. Each point, the mean of at least six tumors. Error bars, ±SE.
approaching the MTD (6, 7, 18, 20 dolastatins, colchicine, FAA, and DMXAA, do so only at doses

marked vascular shutdown within tumors, such as Vinca alkaloids, dolastatins, colchicine, FAA, and DMXAA, do so only at doses approaching the MTD (6, 7, 18, 20).

There are two aspects of the wide therapeutic index that must be explained: First, the occurrence of vascular effects at doses producing little or no classical antitubulin-mediated toxicity, and, second, the devastating effect on tumor vasculature without the undoubted catastrophic toxicity that would ensue if all vasculature of the host animal were similarly affected.

In relation to the first aspect, it is noteworthy that the tubulin-binding agents that are shown to have vascular-damaging activity only at their MTDs, have extensive tissue distribution and prolonged elimination half-lives in vivo. For example, colchicine has a volume of distribution of 700 liters in humans and a terminal elimination half-life of ~20 h (23), whereas the volume of distribution for vinblastine has been measured at 1400–2500 liters, and its terminal elimination half-life was calculated as 30–35 h in humans and 5–7 h in mice (24).

The pharmacokinetic study of ZD6126 in mice indicates a much faster elimination of the active drug NAC. The rapid reversibility of NAC effects, as demonstrated in the endothelial shape-change assay, may also be beneficial. It is possible that a short in vivo exposure to the tubulin-binding agent is adequate to cause rapid damage to tumor vasculature but avoids toxicities because of a prolonged disruption of microtubules in other tissues.

Several factors may contribute to the selective effect on tumor vasculature versus normal vasculature. Unlike normal vasculature, the tumor vascular system contains regions of active angiogenesis. Furthermore, the vasculature of many tumors bears the hallmarks of immaturity and is not well supported by vascular mural cells such as pericytes and smooth muscle cells (25). It is also likely that there are morphological differences within endothelial cells in regions of immaturity and active angiogenesis. A particular feature of endothelial cells in normal, mature vasculature is the presence of a dense peripheral band of actin microfilaments, which is important for the maintenance of endothelial cell structural integrity (17, 26). During angiogenesis associated with wound healing, this feature disappears, and it is likely that at least some endothelial cells in tumor vasculature will also lack this structural element of the cytoskeleton. The role of the dense peripheral band can be modeled in monolayers of endothelial cells in culture because confluent cells display the band, whereas nonconfluent, proliferating cells do not. The ability of ZD6126 to induce dramatic shape change preferentially in nonconfluent HUVECS suggests that immature tumor vasculature may be targeted, at least in part, because of differences in actin cytoskeletal architecture. This susceptibility may be further enhanced in vivo by the lack of mural cells providing mechanical stability to the vasculature. It is also interesting to note that other cell types also contain well-defined actin microfilament structures similar to the endothelial cell-dense peripheral band. For example, platelets exhibit a wheel-like actin structure that appears to be important in the maintenance of a discoid shape in the presence of colchicines (27). An actin-containing structural element, the actin-edge bundle, present in sparse cultures of fibroblasts, is also not dependent on microtubules for its stability (28). Such elements, as well as possible intrinsic resistance to depolymerization of different forms of tubulin, could protect nonendothelial cells from the effects of ZD6126.

Although the relationship of endothelial cell-shape change to the observed vascular shutdown is not fully delineated, it is clear that the rounding of endothelial cells in vivo would compromise vessel integrity.

After the single-dose treatment with ZD6126, a thin viable rim of tumor cells remained adjacent to normal tissue, similar to the pattern of necrosis observed with combretastatin A-4 phosphate. These cells may be supported by adjacent tissue blood vessels and/or vessels within the edge of the tumor that remain unaffected by ZD6126. It has also been suggested (29) that elevated hydrostatic pressure in central regions of the tumor has a role in the vascular collapse observed after combretastatin treatment and, because hydrostatic pressure can drop off rapidly at the tumor edge (30), this offers an explanation for the preservation of blood flow in the periphery and for the survival of a rim of cells.

The clonogenicity of the surviving cells after ZD6126 treatment has been demonstrated and probably leads to the lack of significant growth delay generated by single doses of ZD6126. Similar modest effects of a single dose on tumor growth have been reported for combretastatin A-4 phosphate, both in the same model (31) and in other (32) tumor models. However, because multiple-dose regimens of ZD6126 were tolerated, we

Fig. 9. A. Growth delay induced in established CaNT tumors by a single i.p. dose of ZD6126. ■ control; ●, ZD6126 (125 mg/kg); ▲, ZD6126 (250 mg/kg). Error bars, ± SE. B, growth delay induced in established CaNT tumors by multiple doses (i.p., 125 mg/kg/dose) of ZD6126. •, saline control; ▲, untreated control; ●, ZD6126 daily Monday through Friday for 2 weeks; ○, ZD6126 daily Monday through Friday for 3 weeks. Error bars, ±SE.

Fig. 10. Growth delay induced in established FaDu xenografts by combination therapy. ■, untreated control; ●, ZD6126 alone (125 mg/kg, i.p.); ▲, paclitaxel alone (15 mg/kg, i.p.); ▲, paclitaxel alone (30 mg/kg, i.p.); ◆, the combination of ZD6126 (125 mg/kg, i.p.) with paclitaxel (15 mg/kg, i.p., administered 15 min earlier).
were able to demonstrate potent tumor growth-delay effects. Furthermore, we were able to demonstrate marked enhancement of paclitaxel activity in a human xenograft model, which suggests that combination treatments with ZD6126 may substantially improve the clinical efficacy of taxane-based chemotherapy.

The preclinical studies reported here show that ZD6126 has promise as a novel vascular-targeting agent for the therapy of solid tumors. This compound is currently in Phase I clinical trial.

REFERENCES

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