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Alterations in Daily Sequencing of Axitinib and Fractionated Radiotherapy do not Affect Tumor Growth Inhibition or Pathophysiological Response

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Abstract

A variety of antiangiogenic strategies have proven effective in preclinical tumor models, either as single agents or in combination with radiation. Clinical gains have been relatively modest, however, and questions remain regarding optimal scheduling. The objectives of the current work were to evaluate whether the sequencing of acute treatment critically affects tumor pathophysiological and therapeutic response. Axitinib (Pfizer Global Research & Development), an inhibitor that predominantly targets vascular endothelial growth factor receptors, was administered either before or after each daily radiation fraction in two human prostate xenograft tumor models. Tumors were frozen at sequential times to monitor changes in (1) vascular spacing, (2) pericyte and basement membrane coverage, and (3) hypoxia. Although similar reductions in blood vessel counts were observed with each tumor model, tumor vasculature was not functionally normalized. Instead, tumor hypoxia increased, accompanied by a progressive dissociation of pericytes and basement membranes. Ultimately, tumor growth inhibition was found to be equivalent for each of the combination schedules. These studies illustrate a clear advantage to combining axitinib with fractionated therapy but argue against an acute radiosensitization or radioprotection of either the tumor cells or tumor vasculature. Instead, post- and preirradiation daily drug administration serve equally well in supplementing the response to radiotherapy.

INTRODUCTION

A variety of novel antiangiogenic strategies have been shown to be effective in preclinical and clinical studies over the past 5-10 years. However, such agents alone are not generally believed to be curative and must instead be combined with conventional cancer therapies such as radiation or chemotherapy. An unresolved question in administering such combinations is whether specific scheduling can be devised to optimize treatment response. For this purpose, it is essential to first determine whether specific antiangiogenics themselves serve to compromise tumor blood flow, which would presumably impair response to both radiotherapy and chemotherapy.

Two hypotheses have become almost dogma regarding the effects of antiangiogenic strategies on vessel morphology and function. The first is that antiangiogenics selectively prune inefficient vasculature, resulting in a transiently normalized vascular configuration and improved tumor blood flow (1). The second is that pericyte-coated vessels are more

resistant to therapy and thus produce a net increase in vessel maturity in vessels surviving initial therapy (2). While such statements may be true in response to certain specific VEGF- or VEGFR-inhibiting agents, the pathophysiological effects after treatment with combinations of antiangiogenics and conventional therapy are far less straightforward (3).

The current investigation was undertaken to evaluate the importance of altering the daily sequencing of therapies, specifically the combination of fractionated radiation with axitinib (AG-013736), a receptor tyrosine kinase inhibitor that predominantly inhibits vascular endothelial growth factor receptors (VEGFRs). Axitinib was administered either 1 h before or 1 h after each 2-Gy radiation fraction. Over short times, at least two opposing mechanisms could conceivably play a role. Since inhibitors of VEGFRs have been shown to sensitize endothelial cells to radiation (4,5), administration of axitinib prior to irradiation could lead to an enhancement of radioresponse. Alternatively, since axitinib has also been shown to significantly increase tumor hypoxia, at least over periods of days-weeks (6), treatment prior to irradiation could also increase hypoxia at more acute times, thus decreasing radioresponse. To better understand the underlying pathophysiological mechanisms, temporal alterations in tumor vascular spacing, pericyte and basement membrane coverage, and hypoxia were quantified in two prostate tumor models in response to the alternate schedules. Although combination therapy produced substantial alterations in tumor pathophysiology and tumor response, precise daily sequencing of such combinations was not an important factor.

MATERIALS AND METHODS

Tumor and Animal Models

The DU145 and PC-3 human prostate carcinoma cells were obtained from ATCC and maintained in DMEM (Mediatech-Cellgro, Herndon, VA) and F12K medium (ATCC), respectively, supplemented with 10% fetal bovine serum. A total of 10^7 viable tumor cells were implanted into the left hind legs of NCr nu/nu male mice and grown to tumor volumes of 200-400 mm³. Tumor (including leg) diameters were measured three times per week using a graduated hole template, with opposite nontumor leg diameters subtracted to calculate actual tumor volumes: tumor volume = $\pi/6 \times (\text{tumor leg diameter}^3 - \text{nontumor leg diameter}^3)$. Mice were housed in microisolator cages and given food and water *ad libitum*. Guidelines for the humane treatment of animals were followed as approved by the University Committee on Animal Resources. Animal welfare at the University of Rochester Medical Center is also reviewed and accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, USDA.

Treatments

Axitinib (AG-013736), a receptor kinase inhibitor of VEGFRs in the clinic, was provided by Pfizer Global Research (La Jolla, CA). Nonclinical studies showed that at higher doses (≥ 25 mg/kg), the compound also inhibits platelet-derived growth factor receptors [PDGFRs, IC₅₀ = 0.1 nmol/liter for VEGFR-1, 0.2 nmol/liter for VEGFR-2, 0.1-0.3 nmol/liter for VEGFR-3, and 1.6 nmol/liter for PDGFR β (7)]. Axitinib was administered daily by gavage in a volume of 0.13 ml. Control animals received 0.5% carboxymethylcellulose drug carrier. Irradiations were performed on nonanesthetized mice using a ¹³⁷Cs source operating at 2.4 Gy/min. Mice were confined to plastic jigs with tumor-bearing legs extended through an opening in the side, allowing local irradiations. Fractionated doses were administered in five daily 2-Gy fractions per week (omitting weekends). For combination treatments, axitinib was delivered either 1 h before or 1 h after irradiation. A single oral dose of axitinib markedly suppresses VEGFR-2 phosphorylation in angiogenic vessels xenograft tumors in mice as early as 30 min after administration, and some inhibition remains for at least 7 h (8).

Mice were killed humanely, and tumors were excised and quick-frozen (using liquid nitrogen) at times from 0-18 days after treatment as begun.

DiOC₇ Perfusion Marker and EF5 Hypoxia Marker

To visualize blood vessels open to flow, DiOC₇, an intravascular stain that preferentially stains cells immediately adjacent to the vessels, was injected by tail vein 1 min prior to freezing (9). Localized areas of tumor hypoxia were assessed in 9- μ m frozen sections (one section taken as near as possible to the tumor center for each combination of stains) by immunohistochemical identification of sites of 2-nitroimidazole metabolism (10). A pentafluorinated derivative of etanidazole (EF5) was injected by tail vein (0.2 ml of 10 mM EF5) 1 h before tumor freezing (11) followed by a second dose 15 min later (to maximize EF5 uptake in the xenograft tumors). Regions of high EF5 metabolism were visualized using a Cy3 fluorochrome (Amersham) conjugated to the ELK3-51 antibody, which is extremely specific for the EF5 adducts that form when the drug is incorporated by hypoxic cells (12). Both the EF5 (made by the NCI) and the ELK3-51 were obtained from the University of Pennsylvania Imaging Service Center (C. Koch, Director).

Immunohistochemistry and Image Acquisition

Tumor sections were imaged using a 10 $\times$ objective (Olympus BX40 microscope), digitized (QImaging Retiga 1300C Peltier-cooled, 12-bit digital camera), background-corrected and image-analyzed using Image Pro software (Media Cybernetics, Silver Spring, MD). Twelve-bit grayscale image montages from 16 adjacent microscope fields (encompassing a total area of 21.6 mm²) were automatically acquired and combined digitally for multiple stains (10). First, images of the DiOC₇ were obtained immediately after cryosectioning, then fixed in cold acetone for 10 min. After staining, this section was returned to identical stage coordinates to obtain images of both EF5/Cy3 and an endothelial cell marker (MECA-32, biotinylated primary, 1:100, Pharmingen, San Diego, CA followed by incubation with Vectastain ABC Elite Standard Kit and AEC detection, Vector Laboratories, Burlingame, CA).

Adjacent frozen sections were sliced to visualize combinations of antibodies, generally dual staining of endothelial cells (MECA-32) with markers for either (a) pericytes [PDGFR β , clone APB5, rat anti-mouse, 1:50, eBioscience, San Diego, CA, generally believed to mark a progenitor pericyte population (13)], or (b) vascular basement membrane (type IV collagen, polyclonal rabbit anti-mouse, 1:2000, Chemicon, Temecula, CA). For pericytes, 20- μ m sections were fixed for 10 s in 1% neutral-buffered formalin, washed and covered in PBS, and imaged for DiOC₇. PBS was aspirated, and the sections were fixed in room-temperature acetone for 1 min and air-dried for 30 min before staining. For type IV collagen, 20- μ m sections were fixed in warm acetone for 1 min and air dried for 30 min. Secondary antibody detection was performed using species-specific Alexa Fluor 488 (type IV collagen) or 546 (Ki-67, PDGFR β) (1:500, Molecular Probes, Eugene, OR) combined with MECA-32 using contrasting Alexa Fluor secondary antibodies.

Image Analysis

As described previously (14), tumor blood vessel spacing was determined using a combination of image segmentation and distance map filtering to obtain a spatial sampling of distance filter intensities, which are directly proportional to the distribution of distances to the nearest vessel. These distances (which are dependent on tumor blood vessel spacing) are reflective of the median distances over which oxygen and nutrients must diffuse to reach all cells of the tumor. EF5/Cy3 images were quantified on the basis of mean pixel intensity within a manually drawn area of interest that excludes sectioning artifacts and normal tissue.

Colocalized and thresholded stained images of MECA-32, type IV collagen and PDGFR β were used to obtain overlap between endothelial and pericyte markers. Percentage coverage was defined as the percentage area overlap divided by the percentage area endothelial cells, and percentage dissociation was the percentage pericyte area that did not overlap endothelial cells. Percentage areas of PDGFR β + cells, endothelial cells, and type IV collagen staining were determined using the ImagePro percentage area measurement in combination with thresholded images of positive staining.

Statistical Analysis

Treatments were compared using Student's *t* test or the Mann-Whitney Rank Sum test and were considered significant at $P \leq 0.05$.

RESULTS

Tumor Progression is Significantly Inhibited by Single and Combined Treatments as Well as by Maintenance Axitinib

To gauge the additive benefits of combined therapy, suitable doses of axitinib or radiation were chosen based on earlier results (6) to slow but not entirely stop tumor progression. Single or combined treatments were given over a period of 2-3 weeks, and tumor volumes were measured three times per week as shown in Fig. 1A and B for the PC-3 prostate carcinoma xenografts. For this experiment, axitinib was administered 1 h before irradiation each day. Similar tumor growth curves for the DU145 xenografts have been reported previously and mirror these dose-response relationships for each treatment group (6). For the PC-3 tumors, the effects of either axitinib or radiation on tumor growth suppression were similar (Fig. 1A). By day 10, the tumor volumes after either single treatment were significantly less than for vehicle-treated controls, and the mean volumes after the combination treatment were significantly smaller than for single treatments.

Figure 1B incorporates two additional schedules: 1 week of combination treatment or 1 week of radiation treatment that in each case was followed by maintenance therapy with axitinib alone for an additional 2 weeks. Compared to controls, tumor volumes at day 9 were significantly reduced for the combination-treated tumors ($P = 0.013$) but were not different for tumors initially treated with radiation alone ($P = 0.105$). Over the next 2 weeks of axitinib maintenance therapy, tumor volumes for both treated groups were significantly lower than controls, volumes were not different between treatment groups, and neither of the treated groups demonstrated any further growth progression over the duration of therapy.

Total and Perfused Vessel Spacing Increases Progressively with Tumor Growth in Untreated Controls and Further Compromised by Combination Treatment

We next monitored the effects of tumor growth on vessel spacing in untreated PC-3 tumors (Fig. 2A) as well as the temporal changes after combination therapy (Fig. 2B). Up to a volume of $\sim 600 \text{ mm}^3$, neither total nor perfused vessel spacing varied significantly for controls, but by $\sim 1100 \text{ mm}^3$ both had increased significantly ($P = 0.002$ and 0.002 , respectively; Fig. 2A). At 1400 mm^3 , perfused spacing was also markedly increased compared to the 1100-mm^3 volume ($P = 0.003$).

The tumors summarized in Fig. 2B were treated with the same two treatment schedules shown in Fig. 1B. The first week included either radiation alone or the combination of axitinib plus radiation, and the next 2 weeks included only axitinib maintenance therapy. At the end of the first 4 days of combination treatment, both total and perfused vessel spacing had increased significantly (Fig. 2B) despite the fact that treated tumor volumes were not different from untreated controls at this time (Fig. 1B). Over the next 2-week course of

axitinib alone, perfused spacing remained high and, in some tumors, increased markedly by day 18 (note the large standard deviation in Fig. 2B). Although perfused vessel spacing at day 18 was much higher than in untreated tumors at comparable volumes of 700-900 mm³, total spacing was roughly equivalent. For the schedule beginning with radiation alone, total and perfused vessel spacings after 2 weeks of maintenance therapy were equal to those of tumors initially subjected to combination therapy (Fig. 2B).

Altered Order of Treatment does not Modify Tumor Growth or Vessel Spacing

To determine whether axitinib produced acute alterations in tumor oxygenation, and therefore radiosensitivity, this agent was administered either 1 h before or 1 h after each 2-Gy fraction of radiation for approximately 2 weeks. The alternate schedules suppressed tumor progression almost identically for both the PC-3 (Fig. 3A) and DU145 (Fig. 3B) tumors. Figure 4A and B shows the corresponding alterations in vessel spacing at days 0, 4 and 11 for PC-3 tumors and days 0, 7 and 11 for DU145 tumors. For PC-3 tumors, both perfused and total vessel spacing significantly increased with increasing treatment time at almost all times compared to pretreatment controls. For DU145 tumors, perfused vessel spacing generally increased with treatment, but the response was quite variable at day 11 for the preirradiation axitinib schedule. No significant schedule-dependent differences in either total or perfused vessel spacing were found at any time with either tumor model.

Altered Order of Treatment has Minimal Effects on Pericyte and Basement Membrane Dissociation or Hypoxia

As shown in Fig. 5A, dissociation of PDGFR β + pericytes from blood vessels increased progressively with the axitinib treatments prior to irradiation. For the postirradiation axitinib treatments, dissociation increased more gradually and was not significantly higher than pretreatment controls until day 11 ($P < 0.001$), when its level approached that of the preirradiation scheduling. Large volume controls are also included in Fig. 5A to illustrate the effect of tumor volume on pericyte dissociation. No significant differences were found between small (370 ± 20 mm³) and large (940 ± 160 mm³) untreated tumors ($P = 0.23$).

Dissociation of type IV collagen (Fig. 5B) is reflective of the dissolution of endothelial cells from their surrounding basement membrane. Both pre- and postirradiation schedules resulted in significant increases in what have sometimes been termed basement membrane ghosts (15), but with no significant differences between schedules ($P = 0.393$). For the type IV collagen, dissociation in untreated tumors also increased significantly with growth ($P < 0.001$). The percentages of vessels covered with either type-IV collagen or PDGFR β were for the most part unchanged in relation to pretreated controls at all observation times (Fig. 5C and D). Although PDGFR β coverage was significantly different between schedules at day 7 ($P = 0.006$), no difference remained at day 11. As with the dissociation, PDGFR β coverage for large untreated tumors was reduced in relation to treated tumors for both schedules, perhaps reflecting of a transition to a more mature pericyte phenotype with tumor growth.

Hypoxia in the DU145 tumors, as determined using EF5 hypoxia marker binding, remained constant over the first 4 days of treatment but increased significantly at day 7 ($P = 0.043$) for the preirradiation schedule (Fig. 6). Although hypoxia increased at a slower pace after the postirradiation schedule, overall levels were not significantly different from those for the preirradiation schedule at either 7 or 11 days ($P = 0.19$ and $P = 0.76$, respectively). By day 11, hypoxia levels for both schedules were also essentially equal to the much larger volume controls.

DISCUSSION

Although preclinical studies have demonstrated striking tumor inhibition in response to a wide variety of antiangiogenic drugs, treatment with such agents is not generally expected to result in total tumor eradication. Clinical trials have therefore invariably combined antiangiogenic agents with conventional therapies (16,17). Since antiangiogenics are specifically designed to slow or prevent blood vessel growth, an underlying concern in designing combination therapies is that blood flow may be compromised, thereby inducing resistance to both radiotherapy (through impaired oxygen transport) and chemotherapy (through reduced drug delivery). Despite the potential clinical relevance, relatively few studies have compared or optimally scheduled combination therapies of antiangiogenic agents plus radiotherapy.

Previous workers have generally taken two approaches in devising optimal treatment combinations with radiation. They have contrasted either acute alterations in the daily sequencing of therapies or more long-term changes in sequential scheduling, whereby either one agent or the other is administered over a period of days-weeks. Without detailed information regarding the precise timing, duration and direction of the tumor blood flow response, it is nearly impossible to predict which approach will be the more beneficial. If an antiangiogenic drug induces a rapid, short-term decrease in blood flow, for example, administration after irradiation could be more effective, while the opposite would be true after an initial increase in flow. If the blood flow response is of longer duration or is cyclical in nature, precise daily sequencing may even be irrelevant.

Even relatively small fractions of radiation have been shown to double the VEGF secretion of tumor cells *in vitro*, increasing progressively from 24-72 h after irradiation (4). In addition to effects related to blood flow, antiangiogenics have also been shown to sensitize endothelial cells to radiation by blocking radiation-associated induction of endothelial survival factors such as VEGF, PDGF and basic fibroblast growth factor (4,18,19). Even under the assumption that this mechanism predominates, the design of optimal timing for radiation therapy in relation to antiangiogenics is not trivial, since it is unclear whether the antiangiogenics need be present before, during or shortly after irradiation. If the antiangiogenic agent sensitizes endothelial cells while at the same time altering blood flow, the net effects on tumor radiosensitivity could be unpredictable.

Given the complex mix of interdependent pathophysiological effects, it is not surprising that prior studies investigating treatment scheduling have been contradictory. Although clear benefits have been demonstrated with combinations of radiation and antiangiogenics [see ref. (20) for review], negative results have also been reported (21). The precise effects of alternative combination radiation/antiangiogenic schedules on therapeutic response have been investigated in only a handful of studies, each with different VEGFR or PDGFR inhibitors. SU11657 was found to be more effective when given 1 day before a single dose of 7.5 Gy than 1 day after, a result attributed to a possible normalization of tumor vasculature (22). Zips *et al.* (23) delivered several weeks of PTK787/ZK222584 either before, during or after fractionated irradiation and reported that only the postirradiation schedule proved effective.

Comparing more acute treatment scheduling, no difference was noted when SU5416 or SU6666 was administered either 30 min before or 30 min after each 2-Gy radiation fraction (24), suggesting that significant changes in tumor oxygenation must not have occurred over this period. Williams *et al.* (25) administered ZD6474 either 2 h before or 30 min after the final 2-Gy radiation fraction. Here the preirradiation drug administration was much less

effective than the postirradiation administration, which was attributed to an acute, drug-induced reduction in tumor blood flow.

In a more mechanistic study, Dings *et al.* (26) defined a specific window of increased tumor oxygenation over the first 4 days of treatment with either bevacizumab or anginex; this was also accompanied by reduced vessel counts and increased pericyte coverage. When radiotherapy was initiated within this window, tumor growth delay was significantly enhanced in relation to alternative schedules. Similar results were reported by Winkler *et al.* (3), who combined DC101 with radiation, although their window of increased oxygenation occurred at a later time. Clearly, optimal scheduling can vary not only with the tumor model but also with the specific agent.

Previous work in our laboratory characterized tumor pathophysiological response after 1-3-week regimens of fractionated radiation, axitinib or the combination in DU145 tumors (6). At weekly times, both axitinib and the combination significantly reduced perfused blood vessel counts while increasing tumor hypoxia. Endothelial apoptosis also increased with the combination regimen. These results argue against a treatment-induced functional normalization of the tumor vasculature at the weekly times, although earlier more acute changes in tumor oxygenation could have been overlooked.

The current investigation was designed to extend these studies in two ways. First, tumors were frozen at days 2, 4 and 7 days after initiation of treatment to better characterize early response. Second, alternative combination treatment schedules were contrasted in terms of tumor pathophysiological response and overall growth inhibition. Since the prior work demonstrated a progressive increase in tumor hypoxia in response to axitinib over the first week (6), we anticipated that combination therapies scheduled to begin with axitinib alone would potentially compromise the effectiveness of subsequent radiation therapy. We therefore compared alterations in acute sequencing: radiation delivered 1 h prior to or 1 h after administration of axitinib. The differences in the response to these schedules presumably reflect both acute modifications in oxygen delivery and axitinib-induced endothelial cell radiosensitization.

At the suboptimal doses selected, both fractionated radiation and axitinib somewhat slowed tumor progression in both the DU145 and PC-3 tumor models. In each case, the combination therapy produced a pronounced and sustained growth inhibition. As shown previously, total and perfused vessel spacing increased substantially with tumor growth for untreated tumors and increased for the combination therapy in the absence of tumor growth. Sustained tumor dormancy was also observed after 2 weeks of axitinib maintenance therapy, regardless of whether the initial treatment was radiation alone or the combination. This agrees with previous studies showing extended tumor control when SU11248 maintenance therapy followed either radiation alone, the drug alone, or the combination (18). Although the tumor volume remained unchanged over the entire course of maintenance therapy, perfused vessel spacing progressively increased to levels equal to those of large-volume untreated controls. The initial increase in vessel spacing over days 1-4 could reflect a pruning of redundant, less mature vessels in the treated tumors followed by a continued increase over days 9-18, as the tumor cells progressively outgrow their vasculature. In control tumors, tumor cells also ultimately outgrew or destroyed their vasculature, but at a much larger relative tumor volume, conceivably a point at which angiogenic growth is no longer able to keep pace with rapid tumor cell proliferation.

To summarize, acute alterations in treatment sequencing had no measurable influence on any of the measured pathophysiological indices. Over 2 weeks of treatment, the tumor growth rate was independent of the treatment schedule for both tumor models. Total and

perfused vessel spacing and tumor hypoxia increased progressively with combination therapy, as did dissociation of both immature pericytes (PDGFR β +) and basement membrane (type IV collagen+). None of these indices, however, varied significantly with treatment schedule. Despite the current results, the order of treatment could significantly affect therapeutic response over more extended treatment periods or with agents that induce more rapid alterations in tumor blood flow. Unfortunately, no reliable, inexpensive and convenient methodology exists for optimally scheduling or comparing such combinations. In addition, universal schedules most likely will not apply over a range of different antiangiogenic agents, which could display distinct mechanisms of pathophysiological response.

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FIG. 1.

Increase in tumor volume as a function of days after initiation of treatment. Panel A: PC-3 tumors, single and combination treatments; panel B: PC-3 tumors with altered combination schedules. Panel A: vehicle control (●); 25 mg/kg axitinib (Δ); radiation (5 × 2 Gy/week, ▲); radiation + axitinib (○). Panel B: vehicle control (●); radiation week 1, axitinib weeks 2-3 (■); axitinib + radiation week 1, axitinib weeks 2-3 (□); +, treatment days for radiation; ×, treatment days for axitinib. Points, mean tumor volumes of 7-12 tumors per group; bars, SE.

FIG. 2.

Alterations in vascular spacing with tumor growth or treatment. Temporal changes in total (○) and perfused (●) vessel spacing with: Panel A: tumor growth in untreated tumors; panel B: combination treatments; (○) total vessel spacing, axitinib + radiation week 1, axitinib week 2; (●) perfused vessel spacing, axitinib + radiation week 1; axitinib week 2; (Δ) total vessel spacing, radiation week 1, axitinib week 2; (▲) perfused vessel spacing, radiation week 1, axitinib week 2; (+) treatment days for radiation; (x) treatment days for axitinib. Points, mean vessel spacing from 6-12 tumors per group; bars, SE, *, significant differences in relation to day 0 controls ($P \leq 0.05$).

FIG. 3.

Increase in tumor volume as a function of days after initiation of treatment. Panel A: PC-3 tumors, panel B: DU145 tumors. (○) vehicle controls, (□) axitinib administered 1 h prior to each 2-Gy daily radiation fraction, (■) axitinib administered 1 h after each 2-Gy daily radiation fraction. Points, mean tumor volumes of 8-12 tumors per group; bars, SE.

FIG. 4.

Alterations in vascular spacing with altered treatment scheduling at days 0, 4 and 11 after treatment initiation. Panel A: PC-3 tumors, panel B: DU145 tumors. Open bars: total vessel spacing; solid bars: perfused vessel spacing. Pre-RT, axitinib administered 1 h prior to each 2-Gy daily radiation fraction; post-RT, axitinib administered 1 h after each 2-Gy daily radiation fraction. Bars, mean vessel spacing from 4-8 tumors per group; SE. *, significant differences of treated tumors in relation to day 0 vehicle controls ($P \leq 0.05$).

FIG. 5.

Effect of DU145 tumor treatment scheduling on (panel A), percentage dissociation of PDGFR β pericytes (panel B), percentage dissociation of type IV collagen (panel C), percentage coverage of PDGFR β pericytes, and (panel D), percentage coverage of type IV collagen. (●) vehicle controls, (□) axitinib administered 1 h prior to each 2-Gy daily radiation fraction, (■) axitinib administered 1 h after each 2-Gy daily radiation fraction. Points, mean percentage dissociation or coverage from 5-8 tumors per group; SE, *, significant differences in relation to day 0 vehicle controls ($P \leq 0.05$).

FIG. 6.

Effect of DU145 tumor treatment scheduling on overall tumor hypoxia. (●) vehicle controls, (□) axitinib administered 1 h prior to each 2-Gy daily radiation fraction, (■) axitinib administered 1 h after each 2-Gy daily radiation fraction. Points, mean EF5 intensity from 4-8 tumors per group; SE, *, significant differences in relation to day 0 vehicle controls ($P \leq 0.05$).