[CANCER RESEARCH 56, 964-968, March 1, 1996]

Advances in Brief

Effect of Radiation on Interstitial Fluid Pressure and Oxygenation in a Human Tumor Xenograft[1]

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Abstract

Elevated interstitial fluid pressure (IFP) is a pathophysiological characteristic of most human and experimental tumors and may be responsible, in part, for the poor distribution of blood-borne therapeutic agents and low blood flow rate in tumors. Recent data in cervical carcinomas in patients suggest that fractionated radiation can lower tumor IFP and increase oxygen partial pressure (pO_2) in some patients. The goals of this study were to find the minimum dose of radiation required to modulate IFP and pO_2 and to determine the time course of IFP changes due to radiation in a preclinical model. Xenografts of the LS174T human colon adenocarcinoma were grown in the right flank of nude (BALB/c) mice. IFP and pO_2 were measured before and 24 h after graded doses of irradiation. The mean ± SD initial IFP in untreated tumors was 12.9 ± 0.5 mm Hg (n = 109), and the range was 3.0 to 40.3 mm Hg. The mean ± SD and median initial pO_2 were 20.2 ± 2.4 and 11.9 mm Hg, respectively (n = 37). IFP and pO_2 were independent of tumor size. Fractionated radiation lowered IFP by 2.5 mm Hg when the total dose was 10 or 15 Gy ($P < 0.05$), but IFP did not change in the controls or the 5-Gy radiation group ($P > 0.05$). Irradiation increased the proportion of tumors at higher oxygen tensions when compared to control tumors. The IFP and tumor volumes were followed for up to 10 days after a single dose of 10, 20, or 30 Gy of irradiation. IFP decreased for all treatment groups. The decrease was most significant for the group receiving 30 Gy. On day five following irradiation, the IFP had decreased by 35%. The changes in IFP and pO_2 occurred before any macroscopic changes in tumor volume could be observed. The radiation-induced decrease in IFP could be, in part, responsible for the increased uptake of monoclonal antibodies following single or fractionated radiation that has been reported in the literature.

Introduction

IFP[3] is elevated in most human and experimental tumors studied (1–3).[4, 5] Several studies have shown, in general, that IFP increases with the size of the tumor, is uniformly elevated in the tumor center, and decreases precipitously to normal values at the periphery (1, 3–6). Three factors are believed to contribute to the elevated IFP: (*a*) the lack of a functioning lymphatic system; (*b*) a relatively high permeability and hydraulic conductivity of tumor vasculature; and (*c*) an increased vascular resistance to blood flow (5, 6). The elevated IFP limits the driving force for convection of macromolecules (*e.g.*, MAbs) across the vessel wall and also causes a radially outward fluid flow, which transports these molecules into the surrounding normal tissue (1, 5). The elevated IFP, therefore, may decrease the delivery of macromolecules to tumors (5). Elevated IFP coupled with the high vascular permeability of tumors may also lead to blood flow impairment in tumors.[6]

Roh *et al.* (2) and Znati *et al.*[5] have shown in patients with cervical carcinomas that a decrease in IFP is associated with a positive response to radiation therapy. If irradiation decreases IFP, then irradiated tumors may allow for better distribution of macromolecules in the tumors. In fact, radiation has been shown to increase the uptake of MAbs in tumors (7–11).

Tumor hypoxia has long been recognized as a contributing factor to radiation resistance (12–16). There is also clinical evidence relating the pO_2 of tumors with response to therapy (2, 13, 16). Large single doses of radiation (10–65 Gy) have been shown to decrease the fraction of tumor below radiobiological hypoxia (2.5 mm Hg) within 96 h after irradiation (15, 17, 18). Another study by Goda *et al.* (19) showed that pO_2 decreased initially on day 1 after single doses of 10, 20, or 30 Gy of irradiation, but reoxygenation occurred over the next 2 days. In a recent study of transplanted rat tumors, fractionated radiation improved oxygenation in the first, second, and third week of treatment, but after 4 weeks (45 Gy), oxygenation decreased to levels lower than pretreatment values (20).

Based on the data of Roh *et al.* (2) and Znati *et al.*[5], this study was undertaken to investigate the effect of radiation on the IFP and pO_2 of tumors. IFP and pO_2 were measured before and 24 h after various doses of irradiation to determine the minimum dose of radiation required to affect IFP and pO_2. IFP was also measured for up to 10 days following single doses of radiation to determine the time course of IFP changes due to radiation.

Materials and Methods

Tumor Model. The human colon adenocarcinoma LS174T was obtained from the American Type Culture Collection (Rockville, MD) and was maintained in cell culture. The right flanks of 6–8-week-old female nude (BALB/c) mice (Harlan Sprague-Dawley, Indianapolis, IN) were injected s.c. with 10^7 cells in 0.1 ml of HBSS. Tumor volumes were between 0.3 and 2.5 cm^3.

Radiation. Animals were anesthetized with pentobarbital (50 mg/kg) and irradiated with a cobalt-60 teletherapy source (Atomic Energy of Canada, Ltd., Kanata, Ontario, Canada) at a dose rate of 1.37 Gy/min or with a 6 MeV linear accelerator (Varian, Palo Alto, CA) at a dose rate of 2 Gy/min. Animals were placed on an acrylic sheet with the tumor-bearing limb taped to the acrylic sheet. A 1-cm or a 0.50-cm bolus (for the linear accelerator or cobalt-60 source, respectively) was placed on the tumor, and the tumors were exposed to either fractionated or single-dose irradiation by exposing the whole leg to irradiation. Tumors were irradiated with fractionated radiation to a dose of 5 to 15 Gy in 1 to 3 fractions. Fractionated doses were administered at 24-h

Received 12/27/95; accepted 1/24/96.

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[1] This work was supported by a grant from the Claude Worthington Benedum Foundation (to W. D. B.) and by Outstanding Investigator Grant R35-CA-56591 (to R. K. J.).

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[3] The abbreviations used are: IFP, interstitial fluid pressure; MAb, monoclonal antibody; pO_2, oxygen partial pressure.

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[5] C. A. Znati, K. Karasek, C. Faul, H-D. Roh, Y. Boucher, M. Rosenstein, S. Kalnicki, R. Buchsbaum, A. Chen, W. D. Bloomer, and R. K. Jain. Interstitial fluid pressure changes in cervical carcinomas in patients undergoing radiation therapy, submitted for publication.

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intervals. In the longitudinal experiments, tumors received a single dose of 10, 20, or 30 Gy.

IFP Measurements. IFP was measured using the wick-in-needle technique (2). Five pieces of monofilament nylon from standard surgical suture (6–0 Ethicon) were placed in a 23-gauge hypodermic needle with a 2-mm side hole located 4 mm from the tip. The needle was connected to a pressure transducer (P23ID; Gould Electronics, Cleveland, OH) by polyethylene tubing (PE50) filled with heparinized isotonic saline (70 units/ml). Instrumentation was calibrated against known pressure heads. The needle was placed in the tumor center, and the pressure signal was amplified (Model 11-4113-01; Gould Electronics), digitized (MacLab system; World Precision Instruments, New Haven, CT), and stored on a Macintosh computer. Fluid communication was checked by compressing and decompressing the tubing with a metal clamp. If the pressure before compression, after compression, and after decompression did not differ by more than 10%, then the average pressure of these three readings was calculated.

pO_2 Measurements. Oxygen tension profiles were measured using commercially available 23-gauge hypodermic needle electrodes (Model 737–23; Diamond General, Ann Arbor, MI). The electrodes exhibited a mean oxygen sensitivity of 3.0–4.0 pA/mm Hg when placed in isotonic saline at 37°C. The electrodes were polarized in isotonic saline for 2 h before use by applying a constant voltage of −0.7 V (Chemical Microsensor; Diamond General, Ann Arbor, MI) and calibrated with pure N_2 and 8% O_2/92% N_2, which were bubbled through the isotonic saline. The electrode was introduced to a depth of 1 cm below the tumor surface. After allowing 5 to 10 min for the electrode to reach equilibrium with the tumor microenvironment, it was withdrawn at a constant rate of 25 μm/s using a hydraulic micropositioner (Model 650; David Kopf Instruments, Tujunga, CA). The electrode current, with a sampling rate of 0.67 Hz, was amplified (Chemical Microsensor; Diamond General) and digitized in a manner similar to that of the IFP measurements. Two to four needle tracks were made in each tumor, and the mean and median pO_2 were calculated. Calibrations were within ±5% before and after measurements. The relative frequency of pO_2 was calculated, and histograms were constructed for individual tumors.

Experimental Design. IFP and pO_2 were measured in the same tumors before and 24 h after fractionated or single-dose irradiation. The radiation doses were 5 Gy in one fraction (n = 7), 5 Gy in two equal fractions (n = 11), 10 Gy in two equal fractions (n = 9), and 15 Gy in three equal fractions (n = 6). Fractionated doses were given in 24-h intervals.

To determine the changes in IFP over time, IFP was measured in tumors for up to 10 days after a single dose of 10, 20, or 30 Gy of irradiation. To avoid anesthetizing the same animals every day, each dosage group was divided into two subgroups (n = 9 for each subgroup of 10 Gy, n = 10 for each subgroup of 20 Gy, and n = 10 and 9 for the two subgroups of 30 Gy). All animals were measured prior to irradiation; then one subgroup was measured on days 1, 3, 5, 7, 9 (after irradiation), and the other subgroup was measured on days 2, 4, 6, 8, and 10. Control animals were divided similarly into two subgroups (n = 11 for each subgroup) for IFP measurements. Another control group (n = 8) had volume measurements taken every day, but no IFP measurements.

Data Analysis. All values except median pO_2 are shown as mean ± SEM. Normally distributed data were evaluated using ANOVA, Student's t tests, and ANOVA with repeated measures. The Kruskal-Wallis and Mann-Whitney U test were used for data that were not normally distributed.

Results

Interstitial Fluid Pressure. IFP values were elevated in all tumor xenografts measured but were not dependent on tumor volume ($P > 0.05$). The mean IFP was 12.9 ± 0.5 mm Hg, and average tumor volume was 0.85 ± 0.05 cm^3 (n = 109). The ranges of IFP and volume were 3.0 to 40.3 mm Hg and 0.3 to 2.5 cm^3, respectively. Mean and median pO_2 were 20.2 ± 2.4 and 11.9 mm Hg, respectively (n = 37). Like IFP, the mean and median pO_2 were not dependent upon tumor size ($P > 0.05$). In addition, there was no correlation between IFP and pO_2 ($P > 0.05$; data not shown; Ref. 21).

Fig. 1 shows the effect of fractionated radiation on IFP in LS174T xenografts. IFP was measured 1 day after the final fraction of irradiation. The decrease was dose dependent, with small doses (less than 10 Gy) not affecting the IFP of the tumors. For radiation doses of 10 and 15 Gy (two doses and three doses of 5 Gy, respectively) a decrease in IFP of 2.5 mm Hg was observed ($P < 0.05$).

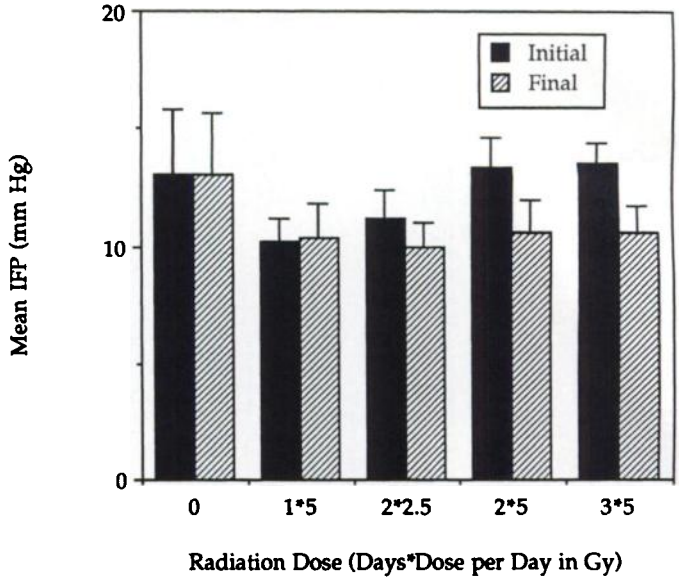

Fig. 1. Effect of radiation on IFP. IFP measurements were taken before irradiation and 24 h after the final dose. Final measurements in control animals were taken 2 days after the initial measurements. There was no significant change in IFP for total radiation doses less than 10 Gy ($P > 0.05$). For the doses that were 10 Gy or above, IFP decreased by the same amount, irrespective of the total dose of irradiation ($P < 0.05$). This indicates a threshold of 10 Gy of irradiation to decrease IFP of these tumors. (For 0 Gy, n = 5 mice; for *1*5* Gy, n = 7 mice; for *2*2.5* Gy, n = 11 mice; for *2*5* Gy, n = 9 mice; for *3*5* Gy, n = 6 mice.)

Oxygenation. Irradiation had no statistically significant effect on mean or median pO_2 at 24 h after the last radiation exposure ($P > 0.05$). In control tumors, pO_2 was measured 2 days after initial measurement. In 60% of irradiated tumors, the fraction of the tumor at low pO_2 decreased, and more of the tumor was at higher pO_2. In contrast, the opposite occurred for the control tumors. Representative data for one control animal and one treated animal (two doses of 5 Gy) are shown in Fig. 2. There was no statistically significant change ($P > 0.05$) in the fraction of tumors with pO_2 readings under 2.5 mm Hg, defined as radiobiological hypoxia (15), at 24 h after fractionated irradiation (Table 1).

Tumor Volume. Irradiation inhibited the growth of the tumors. The increase in tumor volume for all groups of irradiated tumors was not significant ($P > 0.05$), but a significant increase in tumor volume was found for the control group ($P < 0.05$). It is important to note that IFP decreased in irradiated animals in the same period of time for which there was no significant volume change.

Longitudinal Study. IFP was measured before irradiation and for up to 10 days following a single dose of irradiation (10, 20, and 30 Gy). The volume was monitored in control tumors to determine if the IFP measurements affected the growth rate of the tumors. The growth of tumors that had IFP measurements taken every other day did not differ significantly from tumors that were unperturbed by IFP measurements ($P > 0.05$). IFP decreased significantly ($P < 0.005$) for all single doses of irradiation, but the change was most noticeable for the highest dose (30 Gy; Fig. 3). For this case, IFP reached a minimum (35% decrease) on day 5 after radiation, after which IFP began to rise. The two lower doses of radiation caused less decrease in IFP (19 and 23% decrease for 10 and 20 Gy, respectively), and the minimum IFP was reached 2 days earlier than for the tumors treated with 30 Gy (21).

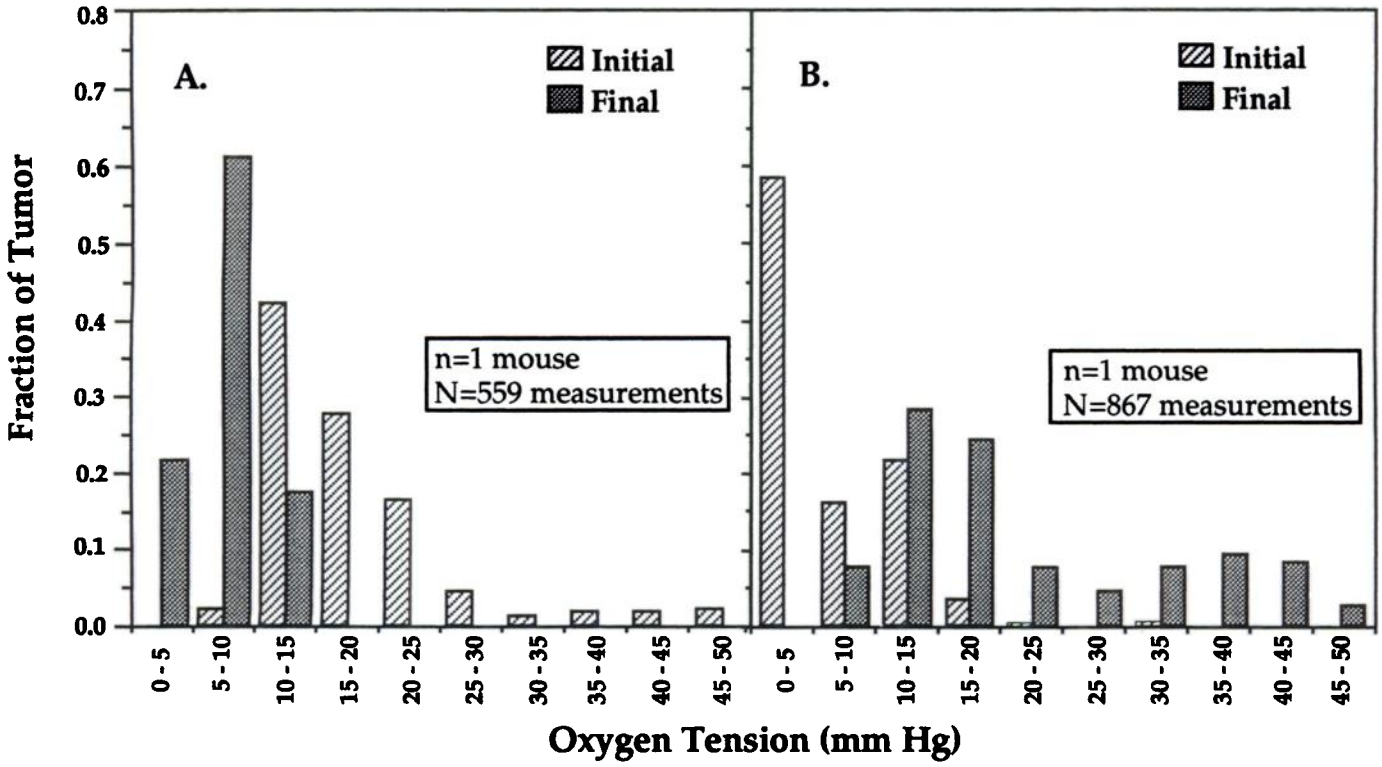

Fig. 2. The change in oxygen distribution for a representative LS174T xenograft after irradiation compared to a representative control tumor. The control tumor has a shift toward lower oxygenation (*A*), while the irradiated tumor (two doses of 5 Gy) has a shift toward higher oxygenation (*B*). One of the 60% of irradiated tumors that exhibited an increase in the proportion of tumor at higher oxygen tensions is shown in *B*, and one of the 80% of control tumors that had a shift to the left in its pO_2 distribution is shown in *A*.

Discussion

The objective of this study was to measure the IFP and pO_2 after various doses of single dose and fractionated radiation. For this xenograft model, IFP was independent of size over a large range of IFP (3.0–40.3 mm Hg) and volume (0.3–2.5 cm^3). In general, the relationship between size and IFP is dependent on the tumor model used. For some transplanted and spontaneous tumors, IFP has been shown to be dependent on size (1, 4, 22–24), but for other tumors, there was no correlation between IFP and size (2, 3).

For the same tumors, there was no correlation between mean or median oxygen tension and tumor size. This may be due to the heterogeneity of the oxygen distribution in tumors or a peculiar characteristic of this tumor line. Several studies have shown a decrease in pO_2 with tumor size (3, 16), although in other studies, no correlations were observed (12, 14). Since there were no correlations of IFP or pO_2 with size, it is reasonable to expect that there would be no correlation between IFP and pO_2 for this tumor model. Similar observations on the lack of correlation between IFP and pO_2 have been reported recently (3).

This study also investigated how radiation affected IFP and pO_2. Our data indicate a threshold for a decrease in IFP at 10 Gy of ionizing radiation. Below 10 Gy, there was no significant change in IFP; however, for doses of 10 Gy and above, the IFP decreased by the same amount (2.5 mm Hg). The change in IFP was due to a cumulative radiation dose and not due to a time effect. Two groups had IFP measured at 2 days after the first radiation fraction. One group received a total dose of 5 Gy, given in 2 fractions of 2.5 Gy, and did not have a statistically significant change in IFP. The other group received a total dose of 10 Gy, given in two fractions of 5 Gy, and this group did have a significant change in IFP. The second IFP measurements were taken at 24 h after irradiation, which preceded macroscopic changes in tumor size.

IFP decreased for all three single doses of irradiation. The decrease began within 2 days of the irradiation and reached a minimum at 3–5

Table 1 *Changes in tumor pO_2 after radiation*

Radiation dose											
Dose (Gy)	Fractions	No. of tumors	Initial mean pO_2[a] (mm Hg)	Final mean pO_2[a] (mm Hg)	P[b]	Initial median pO_2 (mm Hg)	Final median pO_2 (mm Hg)	P[c]	Initial % tumor with $pO_2 < 2.5$ mm Hg[a]	Final % tumor with $pO_2 < 2.5$ mm Hg[a]	P[b]
0	0	5	10 ± 3	8 ± 3	0.44	13	3	0.47	30 ± 15	38 ± 17	0.29
5	1	7	26 ± 6	13 ± 4	0.07	30	6	0.45	22 ± 14	21 ± 8	0.91
5	2	9	18 ± 5	15 ± 4	0.53	7	5	0.47	18 ± 8	30 ± 9	0.10
10	2	9	28 ± 5	41 ± 11	0.20	26	16	0.50	9 ± 5	6 ± 3	0.51
15	3	6	14 ± 5	11 ± 3	0.63	10	5	0.47	20 ± 13	24 ± 7	0.69

[a] Data given as mean ± SEM.
[b] Student's *t* test.
[c] Mann-Whitney *U* test.

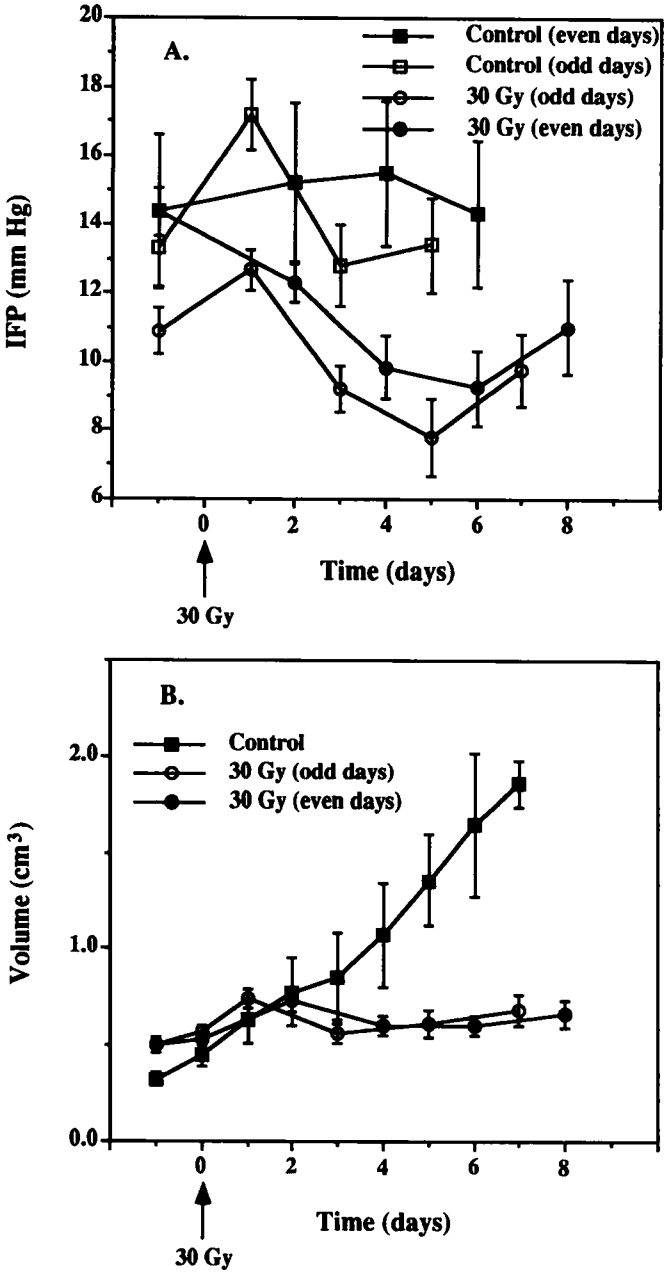

Fig. 3. Effect of a single dose of 30 Gy on the IFP (*A*) and volume (*B*) of LS174T tumors. The IFP decreased by day 2 after irradiation and reached a minimum at day 5. The volume did not start to decrease until day 3 and did not start increasing again until day 7. The change in IFP precedes the change in volume.

days. It is important to note that the change in IFP preceded any change in the growth of the tumor. In fact, compared to tumor size before radiation, the tumor size was not decreased for any of the single radiation doses. In groups treated with a single dose of 10 or 20 Gy, tumor size increased slightly, and in the 30 Gy group, the tumor size remained stable after radiation in the period the IFP measurements were done.

Previous studies have shown reoxygenation in tumors after large single doses (10–65 Gy) of irradiation (15, 17–19). The low doses of radiation given in these experiments had no effect on the mean or median pO_2 of the tumors at 24 h after the final dose of radiation. Important information can still be obtained from these pO_2 measurements, however. Histograms of the proportion of each tumor at given oxygen tensions were constructed. Fig. 2 shows a representative histogram for one control tumor and one irradiated tumor (two doses of 5 Gy). In 60% of the irradiated tumors, the fraction of readings with low oxygen tension decreased; thus, a larger portion of the tumor had higher pO_2 after irradiation. The control tumors showed the opposite effect; 80% of the tumors had a shift to the left in their respective histograms. An increase in oxygen tension may be due to increased blood flow (due to the death of or damage to some of the cells compressing the blood vessels) or reduced oxygen consumption due to death or injury to tumor cells. Reoxygenation has been shown to occur within 6–24 h after a single dose of 10 Gy due to a decrease in consumption and an increase in perfusion (17).

These experiments indicate that the decrease in IFP is due to microscopic changes of the tumor structure rather than the size of the tumor. In light of these results, then, it is not surprising that there was no correlation found between IFP and tumor size. IFP is a balance of the fluid entering a tumor from the blood supply and the exiting of fluid from the tumor by means of the radially outward fluid flow and the tumor vasculature (5). To decrease the IFP, the net accumulation of fluid must decrease. Three potential mechanisms exist that would explain the decrease in IFP after irradiation. The IFP would be lowered if more fluid exited the tumor than entered. An increase in the hydraulic conductivity of the tumor interstitium would increase the fluid flow through the tumor interstitium. We have measured the hydraulic conductivity of the tumor interstitium after radiation and have found that it decreases by an order of magnitude.[7] Since hydraulic conductivity decreases, it cannot explain the decrease in IFP. The radiation may decrease the vascular hydraulic conductivity and vascular permeability in the tumor and thus increase transvascular resistance. This would have the effect of decreasing the amount of fluid that enters the interstitial space. Several investigators have measured the uptake of macromolecules (an indicator of vascular permeability) in irradiated tumors and have found that uptake increased after X-irradiation (7–11, 25, 26). In one study, uptake of macromolecules decreased several days after irradiation (25). A decrease in the venous vascular resistance of the tumor would lead to a decrease in the microvascular pressure, which would lower the IFP (6). An increase in blood flow, which could be due to a decrease in venous vascular resistance, has been shown after irradiation (27–29). This decrease in venous resistance could result from a reduction in compression of venous vessels by cancer cells. From our current knowledge, it appears that the reduction of microvascular pressure is a plausible explanation for the reduction of IFP by irradiation.

Since the distribution of macromolecules in tumors may be affected by the IFP (5), a lower IFP suggests that macromolecules may be penetrating further into the tumor. This may be a possible explanation for the observation that irradiation increases MAb uptake in tumors (7–11). This finding has important implications for combined modality treatment of tumors. If IFP can be lowered by a few fractions of irradiation, treatments utilizing macromolecules may have better success.

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