Measurement of PDT-Induced Hypoxia in Dunning Prostate Tumors by Iodine-123-Iodoazomycin Arabinoside

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Photodynamic therapy (PDT) is known to produce vascular damage in solid tumors resulting in secondary ischemia and tumor cell death from hypoxia. The oxygenation status of both non-treated and PDT-treated Dunning R3327-AT prostate tumors growing in Fischer X Copenhagen rats was investigated with the novel hypoxic marker, ^{123}I-iodoazomycin arabinoside (IAZA). Both qualitative and quantitative data from planar scintigraphy of anesthetized tumor-bearing rats showed increased retention of ^{123}I-IAZA in tumors treated with PDT. Tumor perfusion in the same tumors was measured with ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO). Region of interest analyses revealed an inverse correlation between tumor hypoxia measured by ^{123}I-IAZA and tumor perfusion as measured by ^{99m}Tc-HMPAO (coefficient of correlation, $r = -0.72$). Planar images of 2-mm frozen sections from a large tumor showed ^{123}I-IAZA selectively retained in the region that had been treated with PDT. This and other iodinated azomycin nucleosides, labeled with ^{123}I, show promise for monitoring tumor oxygenation status noninvasively and, in particular, for monitoring the effectiveness of interstitial PDT treatments where perfusion shutdown is a major mechanism of tumor response.

J Nucl Med 1993; 34:405–413

Photodynamic therapy (PDT) is an alternative treatment for localized tumors, especially those known to be refractory to conventional therapies (*1–3*). Effective PDT requires the appropriate combination of three agents: (1) a photosensitizing drug, (2) oxygenation of target cells and (3) an adequate dose of specific-wavelength light (*4,5*). Photofrin®, a porphyrin derivative currently undergoing Phase III clinical trials (*6–8*), is lipophilic and partitions with time into cellular lipid compartments. After excitation with 630 nm light, its excess energy is transferred to molecular oxygen, producing $^1O_2$, which is highly reactive with biomembranes. The resulting membrane damage can produce disruption of mitochondrial oxidative phosphorylation (*9*), membrane leakiness (*10*), lysosomal autolysis (*11*) and endothelial cell damage with the release of vasoactive substances (*12–14*). Studies with animal tumors indicate that the tumor microvasculature is the most sensitive target for PDT, with blood stasis, tumor tissue ischemia and secondary tumor cell hypoxic death as consequential events (*15–18*). Direct tumor cell killing does not appear to be a major mechanism of tumor response for PDT with Photofrin®.

Previous studies from our laboratory have shown that adjuvant bioreductive chemotherapy could potentiate the response of Dunning rat prostate tumors to PDT (*19,20*). We have characterized tumor ischemia and hypoxia induced by Photofrin®-PDT in this animal tumor model by ^{31}P-NMR spectroscopy (*21*), hypoxic sensitizer adduct formation (*20,21*) and tumor perfusion assays with ^{99m}Tc-HMPAO (*22*). These studies showed that PDT-induced tumor ischemia was dependent upon the intensity of the light dose utilized and the time of observation after treatment.

We now report measurements of PDT-induced tumor hypoxia in Dunning R3327-AT tumors with ^{123}I-iodoazomycin arabinoside (IAZA) (*23*). This compound and other nitroimidazoles are metabolically reduced within viable cells at rates inversely proportional to intracellular oxygen concentration and form covalently-linked adducts with cellular molecules (*24*). An Iodine-123 label on this hypoxic marker facilitates the measurement of its adducts by noninvasive nuclear scintigraphy. Our data indicate that ^{123}I-IAZA has potential for monitoring hypoxia induced in solid tumors by PDT. The marker is currently being evaluated as a measure of oxygenation status of human cancers (*25*).

Received Jun. 3, 1992; revision accepted Sept. 30, 1992.

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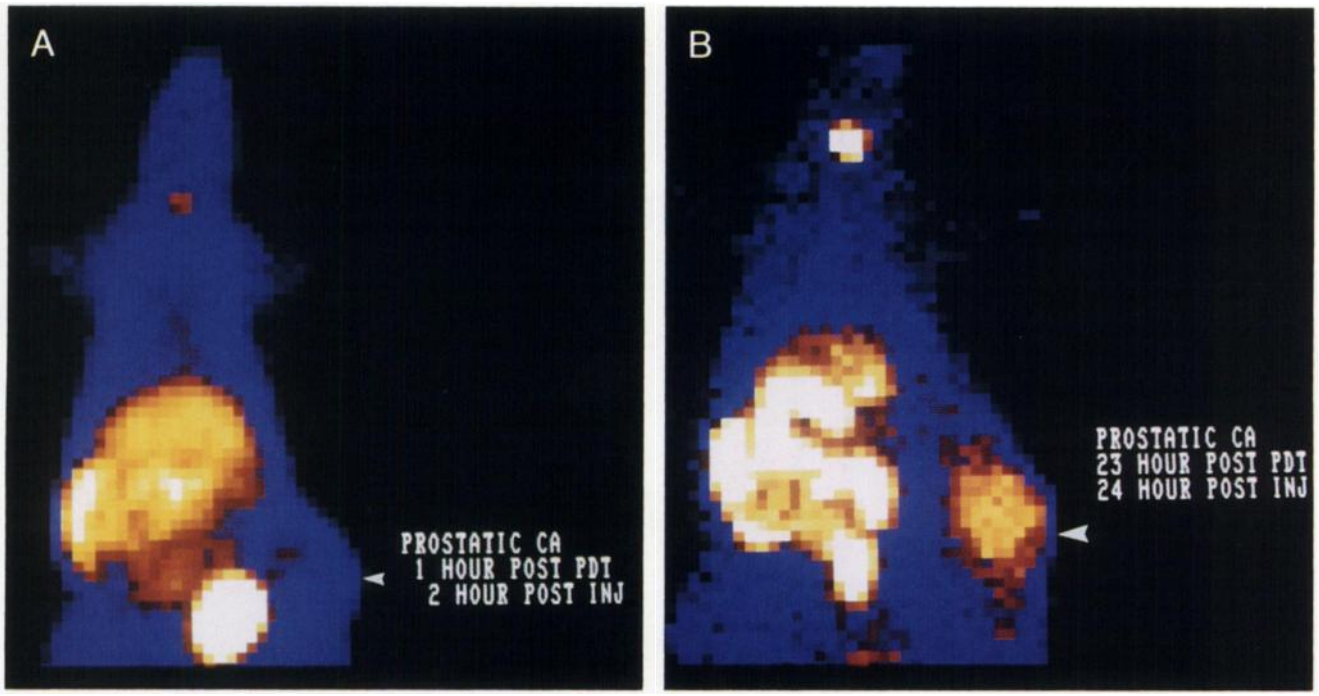

FIGURE 1. Time dependency of ^{123}I-IAZA biodistribution and hypoxia labeling of R3327-AT tumor treated with PDT to 2400 J. (A) Digitized scintigram 2 hr postradiopharmaceutical administration with the activity largely in the liver and urinary tract. (B) Digitized scintigram at 24 hr postradiopharmaceutical administration. Activity is now retained in the unblocked thyroid, hepatobiliary clearance has resulted in a shift of activity from the liver to the bowel, and there is selective retention in the treated tumor.

MATERIALS AND METHODS

Tumor Model

The R3327-AT Dunning prostate tumor displays an anaplastic histology, is aneuploid, hormone-insensitive and grows rapidly in Fischer X Copenhagen rats with doubling times of 2–3 days (*26*). Host rats were bred at the University of Alberta Animal Science Services from breeder stock obtained from Harlen Sprague, Dawley Inc. (Indianapolis, IN) and cared for in accordance with the guidelines of the Canadian Council on Animal Care. Tumor tissue was implanted into rats at least 16 wk of age weighing 200–300 g and tumor growth was measured by procedures previously described (*19*). For our studies, tumors were implanted bilaterally into the flanks of animals. When they reached volumes of ~3 cm^3, one tumor was treated with PDT and the contralateral tumor in each animal served as an untreated control.

Anesthesia

Short-term anesthesia employed for tumor implantation and volume measurements was induced by inhalation of halothane. Prolonged anesthesia required for PDT and scintigraphy was induced with ketamine (12 mg/kg) and xylazene (1 mg/kg) administered via an indwelling tail vein catheter and maintained with a constant infusion of ketamine (25 mg/kg/hr) and xylazene (2.0 mg/kg/hr) (*22*). During prolonged anesthesia, animals were given atropine (0.05 mg/kg intramuscularly) to dry up pulmonary secretions.

Interstitial Photodynamic Therapy

Lyophilized Photofrin® (Lot P89-0089) was obtained from Quadralogic Technologies (Vancouver, Canada). The drug was dissolved in 30 ml of sterile 5% dextrose water to a final concentration of 2.5 mg/ml. Aliquots of 1.5 ml were stored frozen and protected from light until the time of use. Photofrin® was administered at 7.5 mg/kg intravenously 2 hr prior to light exposures. Light at 630 nm, from a coherent CR599 argon-driven dye laser, was split into eight separate beams of equal intensity and directed down 600 μm quartz optical fibers with 1.5 cm cylindrical diffusing tips. Seven fibers were inserted into acetyl plastic needles which had been implanted into tumors 8 mm apart through a template which defined a hexagonal pattern of equilateral triangles. A Laser Guide® power meter measured the output of light from each fiber prior to implantation. One fiber was left in the power meter during treatment to continuously monitor laser light output. Laser light fluence rates were kept constant at ~90 mW/fiber and the time of exposure was adjusted to give different total light doses. A 27 gauge copper-constantan thermal couple (Omega®) was inserted into each tumor to continuously monitor intratumor temperature during light exposures. At light fluence rates of 90 mW/fiber, tumor core temperatures never exceeded 40°C.

Radiopharmaceuticals

Iodine-123-IAZA was prepared on each experiment day in the Faculty of Pharmacy and Pharmaceutical Sciences by procedures previously described (*23*) and transported to the Cross Cancer Institute. This radiolabeled marker was purified by HPLC to a radiochemical purity of 95%-100%. The specific activities of marker samples used in these studies ranged from 20 to 100 GBq/mmol. A purified dry marker was dissolved in sterile 0.9% saline to final activities of 20–80 MBq/ml. One milliliter of the radiolabeled marker was administered via an indwelling tail vein catheter at the time of PDT and the catheter was flushed with 1 ml of 0.9% saline.

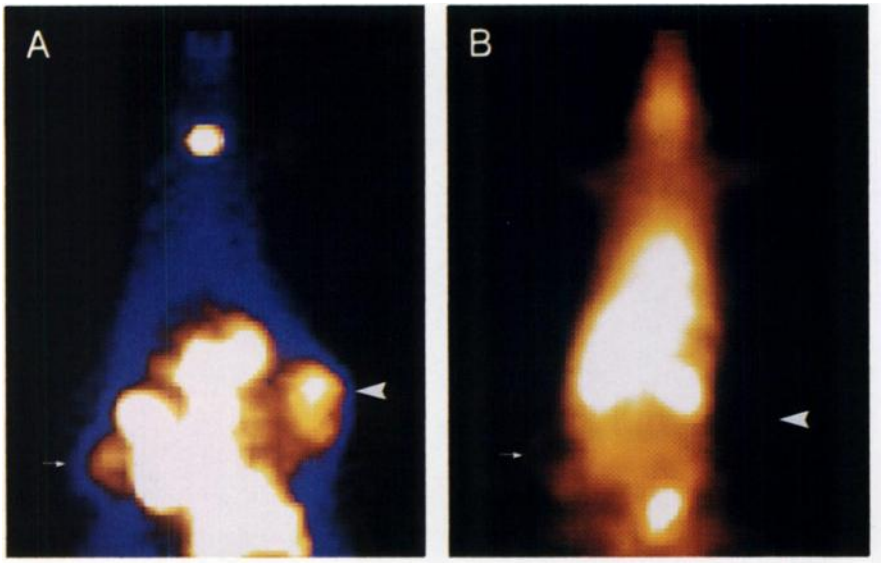

FIGURE 2. Comparison of hypoxia and perfusion imaging of the same rat lying prone on the collimator. (A) Color-coded ^{123}I-IAZA scintigram with the treated tumor (arrowhead) in the left flank and a nontreated tumor (small arrow) in the right flank with a naturally occurring hypoxic center. Activity is also seen within the gastrointestinal tract and thyroid. (B) Technetium-99m-HMPAO scintigram obtained immediately after ^{123}I-IAZA acquisition. The treated tumor (arrowhead) is virtually nonperfused and not evident in the scintigram. The control tumor (small arrow) is moderately perfused with the hypoxic center producing an inverse image to that obtained with ^{123}I-IAZA.

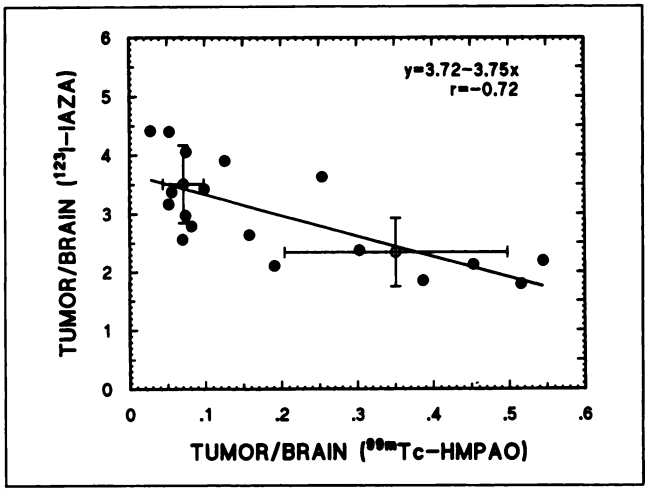

FIGURE 3. Linear regression plot of tumor-to-brain ratios comparing retention of ^{99m}Tc-HMPAO and ^{123}I-IAZA. The open circles and error bars represent the mean and standard deviation of the control and treated tumors. The control group shows the greater deviations.

Technetium-99m-HMPAO was prepared from a kit (Ceretec®, Amersham Int.) by adding 5 ml of second elution ^{99m}Tc-sodium pertechnatate (at 100 MBq/ml) to a lyophilized preparation of HMPAO in stannous chloride. Labeling efficiency of the lipophilic complex was assayed using a two-phase immiscible partition of 0.9% saline and ethyl acetate (*27*). A lipid phase containing >90% of activity was considered acceptable and the preparation was used within 30 min. One milliliter of radiopharmaceutical was administered via the indwelling tail vein catheter and flushed with 1 ml of 0.9% saline.

Scintigraphy

Static planar images were obtained by placing the animals both supine and prone on to the low-energy, high-resolution collimator of a General Electric (Startport®) 400 AC gamma-camera using energy windows of 129–151 keV for ^{99m}Tc and 148–172 for ^{123}I. Images were acquired on a Picker PCS 512 computer using a 128 × 128 matrix. Quantitative data was obtained from defined tissues using the region of interest (ROI) program. To compare ROIs in the different tissues, the pixel

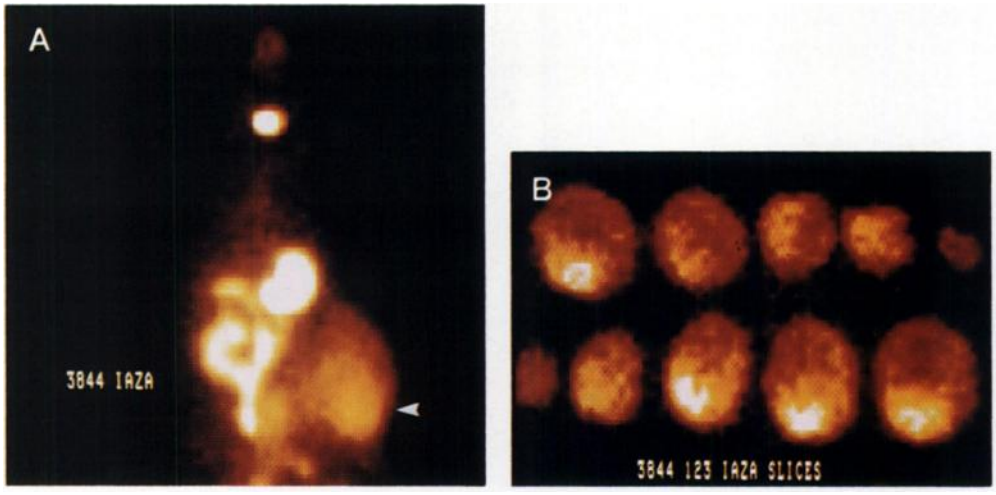

FIGURE 4. (A) Planar ^{123}I-IAZA scintigrams of a rat bearing a large tumor (~8 cm^3) treated with interstitial PDT to the caudal half only (arrowhead). (B) Planar images of 2-mm frozen sections of the tumor in (A) demonstrating selective retention in the PDT-treated caudal portion.

TABLE 1
Nuclear Medicine Assays of PDT Effects on Dunning R3327-AT Tumors

Rat	Light (joules)	^{123}I-IAZA cpm/ROI (Tumor-to-Brain ratio)			^{99m}Tc-HMPAO cpm/ROI (Tumor-to-Brain ratio)		
		Brain	$Tumor_C$	$Tumor_T$	Brain	$Tumor_C$	$Tumor_T$
1	800	41.4 ± 5.0	87.0 ± 3.4 (2.10)	114.7 ± 9.6 (2.77)	6575 ± 210	2972 ± 163 (0.452)	538 ± 62 (0.082)
2	800	117.5 ± 3.5	—	395 ± 27.0 (3.36)	11106 ± 377	—	624 ± 167 (0.056)
3	800	116.8 ± 4.4	—	472.5 ± 29.9 (4.05)	5215 ± 100	—	390 ± 18 (0.075)
4	1600	133 ± 2.5	316.2 ± 16.9 (2.38)	585.5 ± 30.2 (4.40)	7412 ± 161	2245 ± 290 (0.303)	395 ± 49.5 (0.053)
5	1600	151.8 ± 8.1	280.6 ± 28 (1.85)	451 ± 19.7 (2.97)	9874 ± 349	3830 ± 720 (0.388)	728 ± 125 (0.074)
6	1600	97.3 ± 5.3	204 ± 16.0 (2.10)	306.6 ± 11.7 (3.15)	4557 ± 75	880 ± 245 (0.193)	240 ± 13 (0.053)
7	1600	213.6 ± 17.0	776.0 ± 99.8 (3.63)	728.9 ± 52.2 (3.41)	4049 ± 36	1029 ± 128 (0.254)	403 ± 69 (0.100)
8	2400	84.9 ± 3.0	216.8 ± 4.2 (2.55)	223.9 ± 13.9 (2.64)	7104 ± 431	1121 ± 159 (0.158)	496 ± 211 (0.070)
9	2400	60.7 ± 6.3	131.5 ± 4.3 (2.17)	235.5 ± 22.3 (3.88)	5544 ± 69	3018 ± 125 (0.544)	698 ± 58 (0.126)
10	2400	19.5 ± 0.5	32.6 ± 1.4 (1.67)	91.7 ± 1.9 (4.70)	7664 ± 308	3957 ± 365 (0.516)	213 ± 34 (0.028)

area was kept constant (5 × 5 = 25 pixels) and activity measurements from three posterior and three anterior images were averaged to reduce subjective error and error resulting from overlying activity. Retained ^{123}I activity in rat tissues was acquired for 30 min and ^{99m}Tc activity was acquired for 10 min.

The resolution of single-photon emission computed tomography (SPECT) is close to the dimension of the tumors used in this study and consequently this technique provides little information about intratumor distribution of isotopes. Therefore, a larger R3327-AT tumor (~8 cm^3) was treated with PDT to 2400 J using the same hexagonal applicator with seven fibers implanted into the caudad half of the tumor and labeled with ^{123}I-IAZA. Twenty-four hours after treatment, the animal was killed and the tumor excised, frozen in a dry ice and 70% isopropanol slurry and serially sectioned into 2-mm slices. These slices were placed, in proper orientation, directly on to the low-energy, high-resolution collimator of the gamma camera, magnified 2x and planar images were acquired for 30 min on a 128 × 128 computer matrix.

Experimental Protocol

When tumors reached volumes of ~3 cm^3, animals were randomly assigned to one of three groups to be treated with PDT at 800, 1600 or 2400 J, respectively. Only one tumor in each animal was treated with PDT; the contralateral tumor served as a control. Iodine-123-IAZA was administered immediately prior to PDT and marker distribution was confirmed within 2 hr after treatment by acquiring an image over 10 min. The animals were then housed in metabolic isolation cages where they excreted unbound ^{123}I-IAZA and its metabolites for 24 hr. At that time, scintigraphic images (acquisition time 30 min) of ^{123}I were obtained after which animals were immediately given ^{99m}Tc-HMPAO (at a dose that overwhelmed the remaining ^{123}I-IAZA activity) and images of tissue perfusion were acquired for 10 min.

RESULTS

The experiments were performed on seven different days with different batches of ^{123}I-IAZA at different specific activities. On some occasions, studies were performed with excess marker compound which had been prepared for a clinical investigation performed over the same period of time as this study (*25*). The logistics of having available on a given day animals with pairs of tumors of appropriate volume, freshly prepared hypoxic marker, access to the laser facility for PDT treatments and access to the clinical gamma camera (usually after clinic hours) posed some planning difficulties. Volumes of R3327-AT tumors used in this study ranged from 1.8 to 4.9 cm^3 with a mean value of 2.8 ± 1.2 cm^3.

Figure 1 shows planar images of ^{123}I-IAZA distribution in a Fischer X Copenhagen rat with a R3327-AT tumor in the left flank at 2 and 24 hr, respectively, after marker administration and PDT to 2400 J. At 2 hr postinjection, the marker is visible largely in the liver and the urinary tract and in relatively small amounts in both the thyroid and intestinal tract. At 24 hr postinjection, residual activity is observed in the intestine, the thyroid and the PDT-treated tumor. Postmortem measurements of the animal's intestinal tract indicated that >90% of that organ's radioactivity was in the feces. This hypoxic marker is relatively lipophilic (p = 5.0) and is excreted primarily by hepatobiliary processes (*23*).

TABLE 2
Tumor-to-Brain Ratios of ^{123}I-IAZA and ^{99m}Tc-HMPAO in PDT-Treated R3327-AT Tumors

	PDT	n	T/B (^{123}I)	T/B (^{99m}Tc)
1	0	8	2.31 ± 0.6	0.351 ± 0.146
2	800 J	3	3.39 ± 0.64	0.071 ± .013
3	1600 J	4	3.48 ± 0.64	0.070 ± .022
4	2400 J	3	3.74 ± 1.04	0.075 ± .049
5	All PDT-treated	10	3.53 ± 0.70	0.072 ± 0.27

Iodine-123 activity retained in tumor tissue 24 hr after marker administration was indicative of the presence of viable hypoxic tumor cells (*21*) present at short times (up to 4 hr) after marker administration. Hypoxic marker bioreduction rate and adduct formation is a function of marker concentration (*23,24*). Since the hypoxic marker samples prepared on different days had different specific activities and since marker half-life in animals could be variable, the absolute radioactivities measured in tumor ROIs were normalized to the radioactivity within an equal ROI of brain for each host animal. Levels of tissue activity (cpm/ROI) and tumor/brain ratios at 24 hr after marker administration are shown in Table 1 for control (n = 8) and PDT-treated tumors (n = 10). Tumor-to-brain (T/B) ratios were found to vary between 1.67 and 4.70. There was a significant difference in the measured ^{123}I T/B ratio of PDT-treated versus control tumors (p = 0.001) (see Table 2). A light dose-dependent change in marker uptake was not observed in this study. If T/B ratios of ^{123}I-IAZA are representative of tumor hypoxic fractions, it appears that this tumor characteristic is extremely variable in untreated Dunning R3327-AT tumors.

Immediately after the acquisition of the ^{123}I-IAZA data, the anesthetized animals were administered ^{99m}Tc-HMPAO intravenously at levels ~100 times greater than the total ^{123}I activity remaining in the animal. The gamma camera windows were then changed and perfusion images acquired 10 min after the administration of ^{99m}Tc-HMPAO. Iodine-123-IAZA and ^{99m}Tc-HMPAO images obtained from an animal whose left tumor had been treated with PDT to 2400 J are shown in Figure 2. It is obvious that the PDT-treated tumor has retained a greater amount of ^{123}I compared to the untreated tumor and, conversely, there is little or no perfusion marker (^{99m}Tc-HMPAO) in the PDT-treated tumor relative to the non-treated tumor. In fact, one can observe a central perfusion deficit in the control tumor where ^{123}I-IAZA is observed to be selectively retained. Levels of ^{99m}Tc activity (cpm/ROI) in the untreated tumor, the PDT-treated tumor and the brain ROIs (5 × 5 = 25 pixels) are also reported in Table 1. The great excess of ^{99m}Tc activity relative to ^{123}I activity is evident. Again, ROI activity measurements are the averages of three anterior and three posterior measures of cpm within the tissue ROIs. The T/B ratios of HMPAO activity in untreated tumors range from 0.16 to 0.54 with a mean value of 0.35 ± 0.15. This value is consistent with previous studies (*22*). The perfusion of PDT-treated tumors is reduced relative to controls with a mean ^{99m}Tc T/B ratio of 0.07 ± 0.03, (p = 0.00002). Again, with this perfusion assay, a light dose-dependence of the PDT effect was not demonstrated.

In Figure 3, the T/B ratios of ^{123}I-IAZA are plotted versus the T/B ratios of ^{99m}Tc-HMPAO for all of the control and PDT-treated tumors. The line represents a best fit linear regression where T/B (^{123}I) = 3.72 − 3.75 T/B (^{99m}Tc). The correlation coefficient of this data fit is R = −0.72. The open circles in the plot show the mean and standard deviations of T/B ratios for control and PDT-treated tumors. The error bars indicate the large heterogeneity of perfusion and oxygenation status, especially for the control group of untreated Dunning R3327-AT tumors.

Figure 4A shows a planar image of ^{123}I-IAZA within a rat whose tumor (~8 cm^3) was treated with PDT in the caudad region. Thyroid and GI tract labeling is evident along with increased levels of retained hypoxic marker in the caudad portion of the tumor. Figure 4B shows planar images of 2 mm frozen sections of the tumor shown in Figure 4A. These sections were positioned on the gamma-camera to indicate the same orientation of the tumor displayed in Figure 4A (top of sections toward the head and the bottom of the sections toward the tail of the animal). ROI measurements of cpm/25 pixels yielded a ratio of 2 for activity in the caudad compared with the cephalad regions of this tumor. These data of intratumor distribution correspond closely to the non-invasively measured ^{123}I-IAZA retained activity.

DISCUSSION

The data presented here indicate that ^{123}I-IAZA is retained in PDT-treated tumors at levels significantly higher than those measured in untreated control tumors. Unfortunately, while this increase in T/B ratio of ^{123}I is significant (p = 0.001), it is only 1.53 times greater than that observed for the untreated tumors. Furthermore, in this limited study a light dose-dependent increase in T/B ratios of ^{123}I was not observed. Previous studies had indicated the spontaneous hypoxic fraction of Dunning R3327-AT tumors to be quite variable with mean values of between 15%–25% (*21,26*). With ^{3}H-misonidazole as the hypoxic cell marker a 2.4 times increase of retained drug in PDT-treated R3327-AT tumors compared to that of untreated controls was observed (*20,21*). The ratios of bound ^{3}H-MISO in both control and PDT-treated tumors over brain levels were larger than those measured in this study with ^{123}I-IAZA and displayed a light dose dependency up to 2000J of 630 nm light. The extent of tumor

tissue infarction and hypoxic cell induction by PDT is dependent upon photosensitizer dose and activating light dose. Both the formulation and body dose of Photofrin® and the light applicator (7-fiber versus 4-fiber) used in this study were different from those used in our previous studies with ^{3}H-MISO (*20,21*). Consequently, exact numerical agreement cannot be expected.

A major problem with defining a novel technique for estimating tumor oxygenation status is the intrinsic heterogeneity of this tumor property (*21,28*) and the lack of a gold standard of tumor oxygenation to which experimental measurements can be compared. A previous study with this tumor model that compared ^{31}P-NMR spectroscopy with ^{3}H-misonidazole adduct measurements of tumor ischemia showed large variations between assays obtained from tumors in different animals (*21*). Had a tumor model with a very low or no intrinsic hypoxic fraction, such as the R3327-H tumor, been used in these studies, an increase of 4–5-fold in T/B ratios of ^{123}I between severely hypoxic (PDT-treated) and aerobic tumors would be expected (*20*). Other studies with ^{3}H-MISO showed that increased marker association with PDT treated tissue was observed by autoradiography and scintillation counting procedures. Since specimen preparation for autoradiography required multiple tissue washes, we conclude that the majority of radioactive signal in this tissue derives from covalently linked marker. Of course the eflux of unbound marker from tissues whose vasculature has been compromised by PDT would be slower than that from untreated tissue.

Perfusion in the control and treated R3327-AT tumors was measured by ^{99m}Tc-HMPAO techniques 24 hr after PDT treatment. A 5-fold difference was observed between T/B ratios of this marker in untreated compared to PDT-treated tumors. Previous studies (*22*) had shown that the degree of tumor perfusion shutdown after PDT increased up to 8 hr after treatment and then remained shut down for at least 24 hr. Perfusion levels in control tumors of this study were similar to values previously reported (*22*). Nevertheless, perfusion shutdown at 24 hr after PDT treatment should not be directly compared with the values for dose-dependent perfusion shutdown measured with the same marker 1 hr after PDT treatments (*22*). These measures served only to confirm significant perfusion deficits in PDT-treated tumors relative to controls. The relationship between PDT-induced perfusion shutdown and the increase in the fraction of viable hypoxic tumor cells is complex as well. Deficits in tumor blood flow relative to a normal tissue like the brain would not necessarily result in tumor cells with oxygen levels low enough to efficiently bind these marker drugs (*24*). On the other hand, since R3327-AT tumors display an inherent hypoxic fraction, any further compromise of tumor blood flow might be expected to increase its hypoxic fraction. We suggest that studies which measure ^{123}I-IAZA levels in individual tumors before and after PDT treatment would better reflect tumor hypoxia changes by this therapy. Measures performed three days apart should be possible with ^{123}I whose half-life is 13.2 hr. Such studies must await the improved availability of this novel hypoxic marker. Multiple measures of perfusion shutdown in individual tumors with ^{99m}Tc-HMPAO have been performed with the R3327-AT tumors (*22*). The data of Figure 3 suggest that a relationship does exist between ^{123}I-IAZA uptake into Dunning R3327-AT tumors and tumor perfusion as measured by ^{99m}Tc-HMPAO, albeit a complex relationship.

The planar images of frozen tumor sections shown in Figure 4B suggest that ^{123}I-IAZA is retained within the tumor at the site of PDT treatment. While this is encouraging and confirms the ability of this compound to mark hypoxic tissue (*23*), it is the noninvasive measurements of retained tumor radioactivity which would be most useful for monitoring in vivo tumor response to PDT. In this regard, the preliminary data obtained with ^{123}I-IAZA labeling of untreated human tumors (*25*) offer as much encouragement for the successful development of this class of hypoxic marker as do the studies reported in this manuscript.

The field of PDT has seen considerable advances over the past few years. The identification of several new photosensitizing drugs which can be activated at longer wave lengths should improve the feasibility of treating accessible human solid tumors by interstitial PDT procedures. When such photosensitizers are clinically tested, noninvasive assays of PDT-induced tumor response such as ^{123}I-IAZA, ^{99m}Tc-HMPAO and ^{31}P-NMR spectroscopy could prove useful for verifying changes in treated tumor physiology which are believed to be prerequisite steps for successful response and cure of individual tumors.

ACKNOWLEDGMENTS

This research was supported by grants from the Alberta Cancer Board, the Alberta Heritage Foundation for Medical Research and the National Institutes for Health (USA) CA52101. The assistance of Mark Arnfield in maintaining the lasers and of Cindy Johns, Anne Carson and Barbara Haagen in preparing the manuscript is appreciated.

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EDITORIAL

Second Generation Hypoxia Imaging Agents

The contribution by Moore et al. (*1*) comes from the same laboratory that first proposed hypoxia imaging in 1979 (*2*) and describes imaging of both nontreated and photodynamic therapy (PDT) treated prostate tumors in rats with iodoazomycin arabinoside (^{123}I-IAZA). Tumor perfusion in these same tumors was measured with ^{99m}Tc-HMPAO. Part of the clinical interest in PDT involves the development of techniques for the treatment of human prostatic adenocarcinomas (*3*). This therapy results in membrane damage dependent upon the concentration of the photosensitizing drug, the activating light source and the intracellular concentration of oxygen (*4*). If there is insufficient oxygen present, singlet oxygen ($^1O_2$) cannot be produced, and the desired disruption of mitochondrial oxidative phosphorylation, membrane leakiness, lysosomal autolysis and endothelial cell damage are not achieved. One limitation of PDT is that it results in formation of a hypoxic cell fraction within the tumor being treated, which is then resistant to further PDT treatment. Previous work from these authors demonstrated that PDT-induced tumor ischemia was dependent upon the light dose used and the time of observation after the treatment (*3*). The microvasculature was the most sensitive target for PDT and the consequential events after PDT include blood stasis, tumor tissue ischemia and secondary tumor cell hypoxic death. Therefore, measurement of oxygenation status and perfusion within the tumor should be useful in monitoring the potential success of PDT. An iodinated azomycin nucleoside hypoxic cell marker that displayed greater sensitivity to O_2 levels, thus allowing for closer monitoring of the progress of PDT in tumors, would be a valuable contribution.

The report concludes that ^{123}I-IAZA and other iodinated azomycin nucleosides show promise for monitoring tumor oxygenation status, in

Received Dec. 10, 1992; accepted Dec. 10, 1992.
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