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Effects of Pd-bacteriopheophorbide (TOOKAD)-Mediated Photodynamic Therapy on Canine Prostate Pretreated with Ionizing Radiation

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Abstract

The aim of this study was to evaluate the effects of photodynamic therapy (PDT) using a novel palladium bacteriopheophorbide photosensitizer TOOKAD (WST09) on canine prostate that had been pretreated with ionizing radiation. To produce a physiological and anatomical environment in canine prostate similar to that in patients for whom radiotherapy has failed, canine prostates ($n = 4$) were exposed to ionizing radiation (54 Gy) 5 to 6 months prior to interstitial TOOKAD-mediated PDT. Light irradiation (763 nm, 50–200 J/cm at 150 mW/cm from a 1-cm cylindrical diffusing fiber) was delivered during intravenous infusion of TOOKAD at 2 mg/kg over 10 min. Interstitial measurements of tissue oxygen profile (pO_2) and of local light fluence rate were also measured. The prostates were harvested for histological examination 1 week after PDT. The baseline pO_2 of preirradiated prostate was in the range 10–44 mmHg. The changes in relative light fluence rate during PDT ranged from 12 to 43%. The acute lesions were characterized by hemorrhagic necrosis, clearly distinguishable from the radiotherapy-induced pre-existing fibrosis. The lesion size was correlated with light fluence and comparable to that in unirradiated prostate treated with a similar TOOKAD-PDT protocol. There was no noticeable damage to the urethra, bladder or adjacent colon. The preliminary results obtained from a small number of animals indicate that TOOKAD-PDT can effectively ablate prostate pretreated with ionizing radiation, and so it may provide an alternative modality for those prostate cancer patients for whom radiotherapy has failed.

INTRODUCTION

In the United States, prostate cancer is the most commonly diagnosed male cancer and is the second leading cause of cancer death among men (1). Surgery, radiation and hormone therapy or their combinations are available treatment options for the management of localized and advanced disease. Significant side effects, such as impotence, urinary incontinence and injuries to nearby structures, are associated with these procedures (2, 3). Other approaches, such as cryotherapy, hyperthermia, focused ultrasound or focused extracorporeal pyrotherapy, have been developed for the treatment of localized prostate cancer (4–7). However, the available data indicate that the results from these modalities are at best comparable with both radiotherapy and radical prostatectomy (4, 8). An alternative local definitive treatment for organ-confined prostate cancer is thus needed for both primary cancer and recurrent cancer (post-radiotherapy failure).

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Photodynamic therapy (PDT) uses light energy to activate preadministered photosensitizer that is either taken up by cells or in the general circulation. The activation of the photosensitizer results in a series of photochemical reactions that lead to cancer ablation, either through direct cellular destruction or through tissue necrosis as a result of destruction of local vasculature. PDT has been investigated recently as an alternative modality in the treatment of localized prostate cancer. In comparison with conventional modalities such as radiotherapy, chemotherapy and radical prostatectomy, PDT is a localized treatment modality with potentially fewer side effects. In *in vivo* studies, the following photosensitizing drugs have been tested in various prostate (cancer) models: Photofrin (porfimer sodium) (9–11), Puritytin (SnET2, tin ethyl etiopurpurin dichloride) (12, 13), Foscan (mTHPC or Temoporfin, mesatetra[*m*-hydroxyphenyl] chlorin) (14), ALS₂Pc (aluminum disulfonated phthalocyanine) (15), 5-aminolevulinic acid (ALA)-induced PPIX (protoporphyrin IX) (15, 16), Lutrin (Lu-Tex, motexafin lutetium or lutetium texaphyrin) (17, 18), BPD (benzoporphyrin derivative) (19), HYP (hypericin) (20) and, most recently, TOOKAD (Pd-bacteriopheophorbide, or WST09) (21–24). These photosensitizers, activated at optical wavelengths ranging from 630 to 763 nm, affect the prostate tissue through different biological pathways. The results obtained from normal animal prostate or prostate carcinoma studies indicate that PDT, when mediated with the appropriate photosensitizer, can effectively ablate normal or cancerous prostate glands. The application of current preclinical studies to future clinical trials will provide an alternative for prostate cancer patients.

The prostate clinical trial of mTHPC-mediated PDT was carried out in the UK for patients for whom conventional radiotherapy had failed (25). A post-PDT decrease in PSA (prostate-specific antigen) was recorded in 9 of 14 patients. This study suggested that PDT could be suitable for recurrent organ-confined prostate cancer after radiotherapy. Another clinical trial of Lu-Tex-mediated PDT is currently being conducted in the U.S. for a similar group of patients (Study ID: NCI-T99-0042). A recent clinical trial of interstitial PDT using 5-ALA-induced PPIX also showed encouraging results for primary prostate cancer, with patients having a 20–70% reduction of serum PSA levels at 6 weeks after 5-ALA PDT (26).

Our previous study (22–24) showed that TOOKAD-PDT could destroy a clinically significant volume of prostate tissue with preservation of the adjacent tissues (bladder and underlying colon). In our current Phase I clinical trial, TOOKAD-PDT is being given to patients who have locally recurrent cancer after failure of radical radiation treatment. It is known that a full course of ionizing radiation can significantly alter the anatomical and physiological structure of the prostate, in particular inducing the later development of progressive vascular sclerosis and associated fibrosis and glandular atrophy (27). As a result, the tissue optical properties and oxygen profile may be altered and the intrinsic photodynamic sensitivity of the tissue may be altered as well. In turn, these changes may result in different PDT responses, in terms of the type of tissue damage produced and/or in terms of the extent of the PDT-induced effect. Hence, in the present study, we subjected dogs to radical radiation treatment about 6 months prior to PDT and studied the tissue oxygen profile (pO_2) and compared the tissue responses to those measured previously (23) in normal, un-irradiated animals.

MATERIALS AND METHODS

The pre-exposure to ionizing radiation was done at the Veterinary Teaching Hospital of Colorado State University according to a protocol approved by the Colorado State University Animal Care and Use Committee. Surgical, nonsurgical and PDT procedures were performed at the HealthONE Alliance animal surgical facility according to a protocol approved by the Institutional Animal Care and Use Committee.

Radiation Treatment

Four healthy male beagle dogs (Marshall Farms, NY), 2–3 years old and weighing 9.3–16.5 kg, were treated with ionizing radiation in a four-field conformal external-beam arrangement (i.e. dorsal, ventral, left and right laterals). Prior to radiation treatment, the prostate and surrounding structures were imaged by CT scanning (Picker PQ 2000). The image data were networked to a CMS Focus 7200 system (Computerized Medical Systems, Inc.) for 3D treatment planning. The prostate, plus a 1-cm margin, was included in the treatment volume. The treatment volume received a total radiation dose of 54 Gy (2.7 Gy $\times$ 20 fractions, 5 days per week over 4 weeks). This radiotherapy regimen is obtained by a conversion of the biologically effective dose (BED) using a linear-quadratic model (28) and is equivalent to the standard treatment received by prostate cancer patients (typically 1.8–2.0 Gy per fraction in 7–8 weeks for 5 days/week for a total dose of approximately 70 Gy) (29). In addition to dose per fraction, the overall time was also considered. The radiation was delivered using a linear accelerator at 6 MV (Mevatron-6740, Siemens). The animals were anesthetized prior to each irradiation session using Propofol (10 mg/ml; Abbott Laboratories) given i.v. at 4–6 mg/kg. The irradiated animals were kept for 5–6 months, a period within which radiation effects are often seen. It is emphasized that these were cancer-free healthy animals.

Premedication

TOOKAD, as a non-water-soluble drug, is delivered clinically in Chromophore, which is known to induce a drop in blood pressure. Hence, as in our previous canine studies (23) and the current human clinical trials, Benadryl (0.7–1.4 mg/kg, i.v.) and dexamethasone (2 mg per dog, s.c.) were given 24 h before and immediately prior to the photosensitizer infusion.

Surgical and PDT Procedures

Standard sterilization procedures were followed strictly. All surgical instruments were autoclaved and PDT light irradiation probes were sterilized chemically. As an extra precaution, the dogs received antibiotics before and after surgery (Ampicillin, 20 mg/kg, i.m.). Pain control consisted of preoperative and postoperative injections of morphine (0.5–1 mg/kg, s.c.), with long-term control provided by Fentanyl patches. All dogs were prepared for surgery following a standard canine laparotomy procedure (23). A total of four preirradiated dogs received partial-gland light treatment to assess the lesion size and evaluate the efficacy of TOOKAD on preirradiated prostate tissue. Prostate sizes were examined prior to PDT and radiotherapy by CT assessment and were assessed as normal (see Table 1).

TOOKAD[®] (Tookad or WST09; Steba Biotech, France) was administered (2 mg/kg body weight) by slow i.v. infusion over a period of 10 min. Light irradiation was started 4 min after the start of drug infusion since the blood kinetics of TOOKAD is very fast (22) and the intent was to activate the photosensitizer while it was still in the vasculature. A total energy of 50–200 J/cm of diffusing fiber length was delivered at 150 mW/cm over a period of 5.5–22 min (Table 1). There was an approximate 6-min overlap between drug infusion and light irradiation. The light source was a 763-nm diode laser (Ceralas; CeramOptec GmbH of Biolitec AG, Germany). For bilateral lobe treatment, the laser output was coupled directly into a Y-splitter (Ocean Optics), allowing two simultaneous irradiation fields (one in each lobe). The interstitial irradiation was delivered through a cylindrical diffuser fiber (1-cm active tip; Medlight S.A.). Each fiber was placed in the center of the lobe from the ventral to the dorsal side, perpendicular to the prostatic urethra. The active tip was approximately 2 cm away from the bladder neck and the fiber tip end was approximately 1.5 cm away from underlying colon.

Tissue pO_2 Measurements

The prostate tissue oxygen tension (pO_2) was measured immediately prior to PDT using an OxyLite system (Oxford Optronics, Oxford, UK). The precalibrated sensor (operating range up to 100 mmHg) consists of an optic fiber (diameter $\sim 220 \mu\text{m}$) that measures pO_2 by determining the oxygen-dependent fluorescence lifetime of a ruthenium chloride coating on the tip (30). A 22-gauge needle was used to penetrate the capsule and advance the fiber into the prostate. Up to three measurement tracks were obtained for each lobe, in the anterior, mid and posterior sections. To determine the pO_2 baseline of preirradiated prostates ($n = 4$) prior to PDT, the inspired oxygen (FIO_2) was adjusted briefly from 95–98% to room air breathing (21%). The baseline pO_2 of each track was recorded 2–3 min thereafter. The FIO_2 was switched back to 95–98% immediately after pO_2 measurements. The baseline pO_2 of unirradiated prostates of two control dogs (3 and 5 years old and both weighing 21 kg; Marshall Farms) was recorded in the same way. The tissue pO_2 levels of unirradiated prostate and irradiated prostate were compared by a two-sample t test. Statistical significance was assumed for $P < 0.05$.

Dynamics of Light Fluence Rate in Tissue

To monitor the dynamic changes, if any, in the light fluence rate within the preirradiated prostate during PDT treatment, an isotropic optical fiber probe (800 μm in diameter) was placed transurethrally into the midpoint of the prostate urethra and coupled to a photometer (Model 88XL, Photodyne, Westlake Village, CA) (23). Readings of the local light fluence rate were made every 30 s during PDT. The photometer was reset before each PDT session to compensate for ambient light. Since the precise distance between the light source(s) and this detector was unknown, no attempt was made to examine the optical attenuation depth, but the measurements served to monitor the relative changes in fluence rate.

Care after PDT

After surgery the dogs were kept in dimmed ambient lighting for 2–4 h before being transferred to the housing facility. The dogs were placed in metabolic cages for urine collection during the first 24 h after PDT and prior to killing. Urinalysis was performed. The treated animals were killed humanely using a barbiturate overdose at 1 week after PDT.

Histopathological Examination

At necropsy, the prostate and bladder were removed and photographed. The underlying colon section was examined visually for possible PDT damage. Tissue specimens were fixed in 10% neutral buffered formalin. For histological examination, the prostates were dissected from the urinary bladder, transversely sliced into 3-mm blocks, and photographed. Areas of gross lesions were recorded. Prostate blocks were embedded in paraffin for over 24 h. Sections 3 μm thick were cut from each block and stained with hematoxylin and eosin (H&E) and Dyer's Verhoeff variation (31) for assessment of necrosis and fibrosis by light microscopy.

RESULTS

Tissue pO_2

The prostate pO_2 profiles of preirradiated and unirradiated dogs were determined by an interstitial optical sensor at the two different FIO_2 settings. Under pure oxygen breathing ($FIO_2 = 95\text{--}98\%$) and standard anesthesia, pO_2 readings of a total of 22 tracks from four irradiated prostates and 12 tracks from two unirradiated prostates were off the scale of the oxygen sensor, i.e. $\geq 100 \text{ mmHg}$, indicating that both unirradiated and preirradiated prostates became hyperoxygenated. Baseline values with room air breathing ($FIO_2 = 21\%$) for 2–3 min are summarized in Fig. 1 for both irradiated and unirradiated animals. The global histograms

shown include all measured points along all tracks and reveal a right shift in the irradiated group. The median pO_2 values were 38.0 and 27.3 mmHg for irradiated and unirradiated prostates, respectively. The corresponding mean pO_2 values $\pm$ SD were 36.8 ± 15.9 and 27.9 ± 14.2 mmHg, respectively. There was a statistically significant difference (t test, $P < 0.0001$). Over 90% of pO_2 readings from irradiated prostate were greater than 10 mmHg. The majority of hypoxic regions were found near the capsular boundary of the dorsal side.

The prostate pO_2 was directly influenced by FIO_2 . Figure 2 shows a representative response of the irradiated prostate, switching between oxygen and air breathing. Previously low pO_2 regions (e.g. <20 mmHg) became well oxygenated under oxygen breathing. The different time responses to FIO_2 changes were noticed. This might be attributed to oxygen perfusion rate, oxygen consumption rate, FIO_2 level and local vascular density.

Dynamic Changes in Relative Light Fluence Rate

The relative light fluence rate was measured with an isotropic scattering light sensor at the midpoint of the prostate urethra during bilateral or single-lobe TOOKAD-PDT, as shown in Fig. 3. The changes seen during PDT were quite variable and in the range of 12–43% of mean values between different animals. It is likely that probe movement during the measurement could result in some of the larger variations in light fluence measurements.

Observations after PDT

None of the four dogs receiving interstitial PDT had any clinical signs or infection in the 1-week follow-up period after PDT. They all resumed normal spontaneous urination upon recovery from the surgical procedure, with no signs of incontinence or passage of tissue debris or significant macroscopic hematuria. Urinary catheterization was not required in any animal. Urinalysis performed 1–3 h after PDT and 24 h before killing showed traces of blood. These findings were similar to TOOKAD-PDT treatment of unirradiated animals as described previously (23).

Macroscopic Findings

One of the four irradiated dogs received bilateral PDT treatment: 50 J/cm to the left lobe and 100 J/cm to the right lobe. The other three received single-lobe interstitial irradiation: 50 J/cm (one dog) and 200 J/cm (two dogs), with the opposite lobe serving as a control (Table 1). At 1 week, the PDT-induced lesions were characterized in general by acute hemorrhagic necrosis and edema, accompanied by patchy subcapsular hyperemia. The extent of the PDT-induced lesions corresponded well with the total irradiation fluence: The maximum lesion diameter was 12–15 mm for 50 J/cm, 21 mm for 100 J/cm, and 24–28 mm for 200 J/cm (see Table 2). The lesion induced by 200 J/cm crossed the prostate midline in both animals. Figures 4 and 5 show gross and dissected views of the lesions. There was no visually observable damage to the urethra, bladder or underlying colon section.

These macroscopic findings are comparable to those in unirradiated animals. In particular, the lesion sizes are similar, even though in the previous study (23) a 5- or 15-min interval was used between the end of TOOKAD infusion and the start of light treatment. (The reason for changing to the present overlapping of the drug and light administration is that it is now known that the light delivery period should be centered around the peak of the photoactive monomeric photosensitizer concentration in blood and hence putatively should be maximally effective. This overlapping regimen is also now used in the Phase I/II clinical studies.)

Histopathology

Histopathological examination of the prostate after PDT showed chronic fibrotic regions (unaffected by PDT) and necrotic regions (affected by PDT). This indicated that at 5–6 months after radical radiotherapy to the whole prostate and prior to PDT, irradiated prostates underwent severe fibrosis and glandular atrophy. Microscopic examination confirmed that severe necrotic lesions were induced by the TOOKAD-PDT in the fibrotic regions. These lesions were well demarcated and were distinguishable from the chronic fibrotic radiation response. Some extended to the prostate capsule. H&E staining of the PDT-induced lesions confirmed marked hemorrhage, necrosis and atrophy in the glandular ducts and acini and in the interstitial conjunctive tissues as well as the surrounding fibromuscular tissues (see Fig. 6). Dyer's Verhoeff staining for collagen showed loss of stromal connective tissue and total destruction of the acinar collagen in the center of PDT-treated areas (Fig. 7). No urethral lesions were observed in any of the specimens, even though part of urethra was in the PDT treatment zone. These histological findings are indistinguishable from our previous observations in normal, unirradiated canine prostate (23).

DISCUSSION

The normal canine prostate has served as a useful preclinical model for evaluating responses to PDT *in vivo*, since its size and general anatomical structure are similar to those of the human prostate, although it is more cystic while the human prostate is more fibrous and surrounded by a tougher capsule. Canine prostate neoplasia/carcinoma shares many of the features of human prostate cancer and thus is a suitable spontaneous preclinical prostate cancer model (32). Although PDT-related tissue destruction and healing may show some differences between human and canine prostate models, the latter remains the only generally accepted model for studying PDT ablation preclinically. It is particularly relevant in evaluating and optimizing TOOKAD-PDT, for which the clinical intent is to achieve complete prostate destruction, without selectively targeting the malignant components.

It is well known that the efficacy of PDT may be affected by the presence of pre-existing hypoxic tumor regions or by oxygen depletion during the PDT light irradiation (33, 34). In this study, the prostate tissue pO_2 measurements indicated, surprisingly, that irradiated prostates were better oxygenated than unirradiated prostate, even under room air breathing. This may be due to reduced cell metabolism and increased inert components such as collagen (see Fig. 7). The irradiated prostate tissue also became well oxygenated under oxygen breathing, with no hypoxic (<10 mmHg) regions noted.

An early study indicated that some hypoxic regions might exist in human prostate carcinomas (35). However, the vascular targeting of TOOKAD-PDT, with consequent ischemia, should lead to the death of both well-oxygenated and hypoxic cells (36), and a recent study has indicated that a certain level of hypoxia in prostate carcinoma would not significantly affect the PDT outcome with a vascular-acting photosensitizer (20). We have also demonstrated recently in a different tumor model that oxygen breathing can facilitate hyperoxygenation of pre-existing hypoxic regions and improve the secondary vascular effects of PDT (34). Nevertheless, the effectiveness of vascular-acting photosensitizers on hypoxic regions of tumor, including prostate cancer, still needs further elucidation.

Accurate optical dosimetry is an important factor to achieve an accurate lesion volume in the prostate and solid tumors (11, 18, 37, 38). Uniformity of light distribution, deep light penetration, and minimal fluctuation of the light distribution during treatment are desirable. Our previous measurements in normal (unirradiated) dog prostates showed a 36% fluctuation of the mean value during the course of TOOKAD-PDT (23). In this study, the light fluence rate measured at the midpoint of the urethra showed a similar magnitude of fluctuations of 12

to 43% (Fig. 3). This is relatively stable, at least compared, for example, to the results using Photofrin (10, 11), where large variations were typical, probably due to the greater sensitivity to variations in blood flow at the shorter wavelength used in the studies (630 compared to 763 nm for TOOKAD). Nevertheless, the variation is sufficient to necessitate online monitoring during clinical TOOKAD-PDT of the prostate to ensure safety with maximum efficacy and control of lesion size.

Our prior studies of TOOKAD-PDT in the normal dog prostate model showed the ability to destroy prostate tissue by hemorrhagic necrosis reliably and over a substantial volume (23) and confirmed that treatment can be given with a very short drug–light interval.

In this new animal model, despite the severe fibrosis and atrophy caused by prior radical radiotherapy, which is similar to that found in the irradiated normal human prostate gland (39), histopathological examination 1 week after PDT showed essentially identical PDT-induced lesions. Within the limitations of the small number of animals and the use of a single set of PDT treatment parameters, the lesion sizes correspond well to those observed in the nonirradiated canine prostates. This supports the concept that TOOKAD-PDT can be used effectively in patients who have relapsed after radiation therapy, as is now being tested clinically. However, there is likely to be a much greater variability in the prostate tissue between patients for whom radiation treatment has failed (40) than are represented by the dog model, even after irradiation, so that the present conclusions serve only as a guide to the clinical study design. Nevertheless, it is certainly encouraging that TOOKAD-PDT does appear to be effective in tissues that had been irradiated previously.

In addition, no adverse effects were observed on the surrounding tissues, such as bladder and underlying colon/rectum, even when the prostate received up to 200 J/cm interstitial irradiation as long as the PDT light sources are positioned sufficiently far away from these critical structures. Since the surrounding tissues would have received some ionizing radiation dose with the four-field radiation protocol used, this suggests that the good safety profile of TOOKAD-PDT, seen previously in unirradiated dogs, is maintained even after radical radiotherapy. The same is true also for the effect on the urethra: Our prior studies in normal dogs (22, 23) showed that the treatment inflicted minimal structural or functional damage and, upon completion of PDT treatment, the animals could resume spontaneous urination without problems, even when the PDT treatment zone included the prostate urethra.

In summary, TOOKAD-PDT appears to be effective in safely destroying prostate tissue that has previously received a radical radiation therapy dose. The responses are qualitatively and quantitatively very similar to those seen in normal unirradiated canine prostate that have formed the basis for the design of the first Phase I/II clinical trials of this new PDT agent for patients for whom radical radiotherapy has failed. The present findings, although in only a small number of animals and for a single set of PDT treatment conditions, indicate that TOOKAD-PDT is effective in inducing marked hemorrhagic necrosis in previously irradiated tissue, without an apparent increase in risk to normal structures. The next planned phase of these preclinical studies will be to increase the number of animals and range of treatment parameters used. In addition, a recent report has suggested the absence of a significant difference in the prostate cancer incidence between intact and castrated canines (41), so that future work will include both intact and castrated canines with spontaneous prostate cancer and will study the effectiveness and optimization of TOOKAD-PDT in achieving total ablation of the cancerous gland.

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FIG. 1.

Baseline values of prostate tissue pO_2 prior to PDT, measured with room air breathing ($FIO_2 = 21\%$). The numbers of pO_2 readings are 108 points from two unirradiated prostates and 172 points from four irradiated prostates. The global histograms shown that the median pO_2 values were 38.0 and 27.3 mmHg for irradiated and unirradiated prostates, respectively. The corresponding mean $\pm$ SD pO_2 values were 36.8 ± 15.9 and 27.9 ± 14.2 mmHg, respectively.

FIG. 2.

Prostate tissue pO_2 changes under different FIO_2 settings. The data were collected from two single tracks of two irradiated prostates. Arrows indicate the switch of FIO_2 setting from oxygen breathing to room air breathing (track 1) or *vice versa* (track 2).

FIG. 3.

Dynamic light fluence changes during TOOKAD-PDT. Series 1–5 represents each measurement session in different animals. Series 1: left lobe, 50 J/cm, right lobe, 100 J/cm, dog 1; Series 2: right lobe, 100 J/cm, dog 1; Series 3: right lobe, 200 J/cm, dog 2; Series 4: right lobe, 50 J/cm, dog 3; Series 5: left lobe, 200 J/cm, dog 4.

FIG. 4.

Gross view of midline cut and laid open in post-radiotherapy, PDT-treated prostate 1 week after PDT. The prostate received 200 J/cm to the left lobe. The lesion crossed the midline and reached the opposite lobe. The dark zone indicates hemorrhagic necrosis and edema.

FIG. 5.

Dissected view of post-radiotherapy, PDT-treated lobes 1 week after PDT. Top panel, 50 J/cm; middle panel, 100 J/cm; bottom panel, 200 J/cm. The dark zones are hemorrhagic necrosis and indicate the increase in lesion size with light fluence.

FIG. 6. H&E-stained micrographs of PDT-induced lesions in prostate, taken from the center of the lesion and showing total destruction of glandular structure (td), necrosis (n), hemorrhage (h), and residual radiotherapy-induced fibrosis (f). Panel A: without prior radiotherapy; panel B: with prior radiotherapy. Original magnification 40 $\times$.

FIG. 7.

Collagen staining (Dyer's Verhoeff) of the prostate receiving PDT after radiotherapy. Showing clear demarcation between the hemorrhagic necrosis (h) (left) and the unaffected fibrotic regions (f) (right). The necrotic region (n) shows a total loss of collagen; the unaffected fibrotic region shows some dense collagen deposition (c). Original magnification 40 $\times$.

TABLE 1**PDT Treatments**

	Dog 1		Dog 2		Dog 3		Dog 4	
Age (months)	26		24		36		26	
Body weight (kg)	16.1		16.1		9.3		16.5	
Prostate volume ^a (cm ³)	33.7		16.3		17.9		23.4	
Radiotherapy-to-PDT interval (weeks)	22.5		20		22		21	
Delivered light (J/cm)	Right 100	Left 50	Right 200	Left 0	Right 50	Left 0	Right 0	Left 200

^a Measured by CT scan prior to radiation treatment.

TABLE 2

Maximum Dimensions of PDT-Induced Lesion (Necrosis) Measured on Gross Tissue Sections

Light dose (J/cm)	Treated lobe	Lesion size (mm)		
		Anterior-posterior	Lateral	Ventral-dorsal
50	Left	15	11	11
50	Right	12	9	10
100	Right	21	19	14
200	Left	28	25	18
200	Right	24	23	>12