Photodynamic Therapy in the Canine Prostate Using Motexafin Lutetium

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ABSTRACT

Our purpose was to determine the feasibility of comprehensive treatment of the canine prostate with photodynamic therapy (PDT) using motexafin lutetium (Lu-Tex) and to evaluate the toxicity and tissue effects associated with this treatment. Twenty-five adult male beagles with normal prostate glands were given an i.v. injection of the second-generation photosensitizer Lu-Tex (2–6 mg/kg). An additional two dogs were used as controls and did not receive any photosensitizing drug. All 27 dogs underwent laparotomy to expose the prostate. Three hours postinjection, a total dose of 75–150 J/cm of 732 nm laser light was delivered interstitially and/or transurethrally to the prostate via cylindrical diffusing fibers. Dogs were euthanized between 2 days and 3 months after PDT. All subjects were monitored for clinical evidence of toxicity. Specimens were examined macroscopically and microscopically to characterize the tissue reaction and assess extent of tissue effect as a result of treatment. Interstitial and/or transurethral PDT were successfully delivered in all dogs with no perioperative complications. No clinical evidence of acute urinary obstruction or rectal bleeding was noted. At all dose levels, macroscopic and microscopic evaluation revealed a prostatic tissue reaction characterized initially (within 48 h) by inflammation and necrosis followed by fibrosis and glandular epithelial atrophy. Comprehensive treatment of the entire prostate could be achieved using the interstitial alone approach or combined transurethral and interstitial approach. The transurethral alone approach did not result in complete coverage of the prostate. Dogs receiving transurethral or combined interstitial and transurethral treatment developed erythema and urethral epithelial disruption at all dose levels. Those receiving combined treatment at the highest dose level (Lu-Tex 6 mg/kg, 150 J/cm light) developed urethral fistulae and pyelonephritis. Dogs treated with the interstitial alone approach were found to have the least amount of urethral damage. Comprehensive treatment of the canine prostate with Lu-Tex PDT is feasible using an interstitial alone or combined interstitial and transurethral approach. The interstitial alone technique results in the least amount of toxicity. The prostatic tissue reaction to treatment is characterized by initial inflammation and necrosis followed by fibrosis and glandular epithelial atrophy.

INTRODUCTION

PDT2 is a treatment modality using light of an appropriate wavelength to activate a photosensitizer in the presence of oxygen, resulting in localized tissue necrosis. PDT has been approved by the Food and Drug Administration for the treatment of selected patients with esophageal cancer and lung cancer (1). Other potential applications and techniques for PDT are being actively explored in the clinic (2–7).

The placement of optical fibers directly into a tumor or organ (interstitial light delivery) is likely the most suitable method for the volumetric treatment of bulk tumor using PDT. Interstitial light delivery to the prostate gland is potentially feasible based on existing techniques used for radioactive implants (8). Clinically, PDT has potential as a treatment for primary localized prostate cancer or for locally recurrent disease for which treatment options are limited (9). Several preclinical studies have evaluated the feasibility of delivering PDT via an interstitial approach in a canine model (10–13). Light-diffusing fibers were introduced directly into the prostate gland and/or via the urethra to deliver the light to the gland. To date, however, no study has directly addressed whether PDT has the potential for comprehensively treating the prostate gland. This issue is important if one wishes to treat prostate cancer (14).

Various photosensitizers have been used for PDT of the prostate gland in preclinical studies. The first-generation photosensitizer, Photofrin, has been shown to cause glandular necrosis in the canine prostate (10). There are several limitations for the use of Photofrin-mediated PDT in the prostate gland. Photofrin is activated by 630 nm light, which has a limited depth of penetration into tissues and is absorbed by hemoglobin. This is potentially a significant problem in the prostate where placement of interstitial needles may lead to bleeding within the

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2The abbreviations used are: PDT, photodynamic therapy; Lu-Tex, motexafin lutetium; mTHPC, meso-tetra(m-hydroxyphenyl)chlorin.
tissues. Furthermore, Photofrin is a partially purified mixture of porphyrin monomers and oligomers and is clinically associated with prolonged (6–8 weeks) skin photosensitivity.

Second-generation photosensitizers may have some advantages for interstitial prostate PDT compared with Photofrin. In general, the second-generation photosensitizers are pure compounds and are associated with shorter skin photosensitivity. Many of these compounds are activated by longer wavelengths of light; thus, the treatment effect may have deeper tissue penetration. Lu-Tex is a second-generation photosensitizer with reported efficacy in murine tumor models (15, 16) and human clinical trials (17). Lu-Tex is a tripyrrolic pentaaza-expanded porphyrin with an absorption band at 732 nm. This longer wavelength of activating light in the far red range may permit more optimal interstitial light delivery than other wavelengths because of greater depth of penetration. Another advantage of far red light is that there is less absorption of light by hemoglobin compared with shorter wavelengths. Skin photosensitivity is relatively minor, with Lu-Tex lasting ~24–48 h. These features support the investigation of Lu-Tex as a photosensitizer for interstitial PDT of the prostate gland.

In this study, we evaluated Lu-Tex-mediated PDT of the prostate in a canine model. The primary end points of this study were: (a) to determine the feasibility of comprehensive treatment of the entire prostate gland using an interstitial, transurethral, or combined interstitial and transurethral light delivery approach; (b) to determine the toxicities associated with Lu-Tex PDT in the canine prostate; and (c) to characterize the histological effect of Lu-Tex PDT on prostatic tissue. The results of this study form the basis on which a future human trial will be initiated.

MATERIALS AND METHODS

Animals, Anesthesia, and Surgical Procedure. Experiments were conducted at the University of Pennsylvania under a protocol approved by the Institutional Animal Care and Use Committee. A total of 27 adult male beagles weighing between 8.8 and 15.0 kg (median, 12.0 kg) were used. All dogs were 1–3 years old. Six dogs were from Harlan Sprague Dawley (Indianapolis, IN), 6 from Marshall Farms (North Rose, NY), 11 from Summit Ridge Farm (Susquehanna, PA), 2 from Ridglan Farms, Inc. (Mount Horeb, WI), and 2 from White Eagle Laboratories, Inc. (Doylestown, PA). Dogs were quarantined for 2 weeks. All dogs received complete blood counts, blood chemistry, and fecal testing to ensure their health. All were vaccinated with Leptospira canicola-icterohaemorrhagiae bacterin and canine distemper/adenovirus type/parainfluenza/parvovirus vaccine from Fort Dodge Laboratories, Inc. (Fort Dodge, IA). All surgical procedures were performed at a University of Pennsylvania animal surgical facility using standard sterile surgical technique.

Biopsies of the prostate were performed under sedation (i.v. Propofol 10 mg/ml to effect) using transabdominal ultrasound-guided 14-gauge biopsy needles (Precision Cut; Becton Dickinson, Rutherford, NJ) 2– 4 weeks preceding Lu-Tex administration. All prostates were sized and assessed as normal. The dogs were premedicated with morphine sulfate, atropine, and acepromazine before surgery and PDT. Anesthesia was induced with i.v. thioental and maintained with 2% isoflurane inhalation via an endotracheal tube. All dogs were monitored by electrocardiography, indirect blood pressure, rectal temperature probe, and pulse oximetry. A preoperative colonoscopy and cystoscopy were performed to clinically assess the genitourinary and gastrointestinal tracts preceding PDT. Each dog was then placed in dorsal recumbency, and the skin was prepared for surgery using standard aseptic technique. A heating pad was used to maintain body temperature. A midline incision was made through the skin extending from the umbilicus to the pubis symphysis and through the linea alba. The connective tissue and fat were dissected to expose the prostate gland (Fig. 1).

Photodynamic Therapy. A total of 27 dogs were treated. Lu-Tex (Pharmacyclics, Inc., Sunnyvale, CA) was administered (2 or 6 mg/kg i.v.) 3 h prior to light administration. A 3-h drug-light interval was chosen because preclinical studies in other model systems demonstrated antitumor efficacy with this timing (15–17). Two light sources were used during the course of this study. In the first 15 dogs, a KTP/532 laser pumping a model 630 XP Dye Module (LaserScope, Inc., San Jose, CA) tuned to emit 732 nm (2 W maximum power) was
used. For the remaining 12 dogs, a diode laser, model 730 (2 W maximum power; Diomed, Ltd., Cambridge, United Kingdom, kindly provided by Pharmacyclics, Inc.) was used. A 2.5-cm cylindrical diffusing fiber with an outside diameter of 800 μm (Rare Earth Medical, Inc., West Yarmouth, MA) was used for light delivery. All fibers were calibrated before and after the procedure using an integrating sphere (Diomed, Ltd.). The transmission efficiency of each fiber, defined as the ratio between light output (as measured from the integrating sphere) and input, was found to be between 58 and 61%. No changes in efficiency were noted between pre- and posttreatment measurements.

After the prostate was exposed, the diffusing fibers were placed in the gland. Interstitial placement of the diffusing fibers was performed using a custom-made polyurethane template with evenly spaced holes which was attached to a pole and centered over the prostate (Fig. 2). Plastic 17-gauge needles (Best Industries, Inc., Springfield, VA) containing metal trocars were placed in parallel at the desired locations in the prostate through the template. Each needle was presoaked in heparin (Heparin Lock Flush Solution; Abbott Laboratories, Chicago, IL) to help prevent thrombus formation around the needle as had been noted in a previous report by Chen et al. (18). The needles were then pushed through the prostatic tissue from a ventral to dorsal direction until the tip could be felt on the opposite side. The trocars were removed and replaced with the light diffusers. There was initial concern that the opaque plastic needles might reduce light output from the cylindrical diffusing tips. Output was therefore measured in an integrating sphere with a bare fiber and a fiber sheathed in a needle. Transmission of light through the opaque plastic needles was 95% as compared with that of the bare fibers. For light fluence/fluence rate specification, output was specified as output from the bare fiber.

Transurethral treatments were performed by placing the diffusing fiber into a clear plastic 5 French 15-inch feeding tube (Premature Infant Feeding Tube; CR Bard, Inc., Covington, GA) and inserting the tube in the urethra until the fiber tip reached just below the bladder neck.

The light fluence was prescribed based on the unit length of radial diffusing fiber (J/cm). The dogs received total fluences ranging from 75 to 150 J/cm at a fluence rate of 75 or 150 mW/cm. The temperature of the prostate during light delivery was monitored (~5 mm from the light source) using a thermal microprobe (Physitemp Instruments, Clifton, NJ).

Table 1 summarizes the treatment delivered to all dogs. Two control dogs were treated with light alone and received no Lu-Tex. When comprehensive treatment of the entire prostate was attempted, the number of interstitial sites used ranged from four to six depending on the size of the prostate gland.

Postoperatively, dogs were observed by a veterinarian for signs of pain/distress or urinary symptoms twice daily for 1 week and once daily thereafter. Butorphenol tartrate (10 mg/ml) was administered 0.4 mg/kg i.v. or i.m. as needed. Selected dogs underwent cystoscopy and colonoscopy days to weeks after PDT to document any visible urethral or rectal toxicity. Dogs were sacrificed from 2 days to 3 months after PDT with i.v. pentobarbital sodium (389 mg/ml) 1 ml/3 kg.

### Table 1: Treatment schema

<table>
<thead>
<tr>
<th>Lu-Tex dose (mg/kg)</th>
<th>Light dose (J/cm)</th>
<th>IT+</th>
<th>IT+</th>
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</tr>
<tr>
<td>2</td>
<td>100</td>
<td>100</td>
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* IT, interstitial light delivery; TU, transurethral light delivery; IT+TU, interstitial and transurethral light delivery.
Photodynamic Therapy in the Canine Prostate

Pathology Review. At necropsy, the bladder, prostate, and rectum were removed and fixed en bloc in 10% neutral buffered formalin. The prostate and urinary bladder were dissected from the rectum, taking care to observe adhesions or evidence of gross injury to the organs and surrounding connective tissues. The urinary bladder and prostate were divided serially in 3–4-mm blocks, photographed, and embedded in paraffin for histological examination. Sections 6 µm thick were stained with H&E and examined to determine the presence of necrosis, hemorrhage, fibrosis, glandular atrophy, inflammation, and other abnormalities.

RESULTS

Lu-Tex Administration and Toxicity. No evidence of skin photosensitivity was observed during the follow-up period of any animal. All dogs were kept in normally illuminated cages in an approved animal facility, although they were not exposed to direct sunlight. An apparent allergic reaction to the photosensitizer injection was noted in 10 of 14 dogs receiving 6 mg/kg Lu-Tex. This reaction was manifested by generalized hives as well as muzzle and paw edema. No respiratory problems were encountered. However, 5 dogs briefly required i.v. fluids to maintain mean blood pressure >60 mm Hg at the time of these reactions. Only 5 of 11 dogs receiving 2 mg/kg Lu-Tex experienced this reaction. None of these dogs required any specific treatment as a result of the reaction.

Clinical Results and Toxicity. In the initial experiments, four dogs were treated (three interstitial alone and one transurethral alone light source arrangement) with Lu-Tex 6 mg/kg i.v. followed 3 h later by light delivery with a fluence of 75 J/cm and fluence rate of 150 mW/cm (Table 1). The goals of these initial experiments were to identify the clinical tolerance of this dose of drug and light within a 2–3-week time frame and to evaluate the zone of necrosis around both interstitial and transurethral treatment fibers. This study showed that the zone of tissue damage surrounding the fibers was irregular but ~1.2 cm in diameter. Given that the diameter of the prostates were significantly larger than the zone of tissue damage created by a single fiber, it was concluded from these studies that transurethral light delivery alone would not achieve the goal of comprehensive treatment of the entire prostate gland. Our initial measurements of the zone of necrosis, however, suggested that interstitial fiber spacing of 1 cm would allow for comprehensive treatment of the gland at this dose level of light.

Based on these initial results, the light fluence was escalated to 150 J/cm at a fluence rate of 150 mW/cm and the Lu-Tex dose remained at 6 mg/kg. The next experiments were designed to evaluate the acute (2 days after PDT) clinical and histological effects from this increased light fluence. Three dogs were treated with a combination of interstitial and transurethral light delivery in an attempt to illuminate the entire gland. The fibers were spaced 1 cm apart cranio-caudally and were no more than 5 mm away from the capsule edge. Immediately after PDT, the gland looked dark and dusky. No acute clinical toxicities were observed. In addition, two dogs were treated with a single interstitial fiber on either side of the gland to assess the zone of tissue necrosis at the higher light dose. Another dog was also treated with a single transurethral fiber to assess the same effect. In each of these dogs, the diameter of necrosis (1.2–1.5 cm) was similar to that in the dogs treated with the lower dose of light.

Additional experiments were designed to evaluate the 3-month clinical and histological effects of the combined transurethral and interstitial light delivery. Four dogs were treated with the combined approach to a total light fluence of 150 J/cm. Two dogs received the light at a fluence rate of 75 mW/cm and two received light at a fluence rate of 150 mW/cm. Immediately after treatment, the prostate looked dark and dusky. All four dogs developed peritonitis (confirmed on necropsy) and either died or were euthanized 3 days to 7 weeks after therapy. Complete, severe, diffuse necrosis of the prostate was noted with complete destruction of the urethra in each dog.

Based on these results, the doses of Lu-Tex and light were decreased. A total of 11 dogs were treated with Lu-Tex 2 mg/kg, with a light fluence of 100 J/cm at a fluence rate of 150 mW/cm. Six dogs received interstitial alone treatment, three dogs received transurethral alone treatment, and two dogs received combined interstitial and transurethral treatment. These dogs were followed clinically from 2 days to 3 months after PDT. No acute or long-term clinical toxicities were observed.

Postoperatively, 24 dogs required one dose of butorphenol for pain. Two dogs required two doses, and one dog required four doses. All dogs were able to urinate and defecate spontaneously after the procedure (on the same day as the surgery). Three dogs had urinary dribbling postoperatively. Each of those dogs received both transurethral and interstitial treatments.

Endoscopic Findings. A total of 10 dogs underwent endoscopic evaluation at various intervals after PDT ranging from 2 days to 10 weeks. Colonoscopies were performed at 2 days to 4 weeks after treatment. No gross rectal abnormalities related to the PDT treatment were noted in any animals. Cystoscopies were also performed at 4 days to 10 weeks after treatment. No gross bladder abnormalities related to PDT treatment were noted. Results of cystoscopic evaluation of the urethras were as follows: (a) two dogs received a transurethral only treatment using either 6 mg/kg Lu-Tex and 150 J/cm light or 2 mg/kg Lu-Tex and 100 J/cm light. At 1 week postoperatively, erythema, hyperemia, and mild necrosis was noted within the lumen of the urethra. By 3 weeks only residual hyperemia remained, and by 5 weeks it had nearly completely resolved. The final cystoscopy performed at 7 weeks was normal with no evidence of stricture; (b) two dogs received interstitial only treatment at 2 mg/kg and 100 J/cm light. Cystoscopies at 1 week revealed mild erythema, edema and minimal necrosis within the urethral lumen. At 4 weeks only mild erythema remained, and at 8 weeks the urethra was normal with no evidence of stricture; (c) three dogs received both interstitial and transurethral treatment at 6 mg/kg and 150 J/cm light. This group had severe erythema and necrosis within the urethral lumen, which persisted at 4 weeks postoperatively. No further endoscopies were performed because each of these dogs died before their next scheduled endoscopy due to treatment complications; (d) two dogs received both interstitial and transurethral treatment at 2 mg/kg and 100 J/cm. This group had erythema, edema, and necrosis at 1 week, with persistent erythema and edema found at 4 weeks. Healing was noted by 7 weeks, although one of those two dogs suffered from a persistent urethral stricture; (e) a light control animal treated with interstitial and transurethral light...
(100 J/cm) but no Lu-Tex had a completely normal cystoscopy 1 week after treatment.

Dogs were scheduled to be euthanized at ~2 days, 2 weeks, or 3 months after treatment. All but four animals were euthanized as scheduled. The four animals that died prematurely were scheduled to be followed for 3 months in an effort to evaluate for long-term toxicity. All four were treated with a combination of interstitial and transurethral light delivery at the highest dose level (6 mg/kg and 150 J/cm) as described above. One dog was found dead in its cage 3 weeks postoperatively, whereas the other three developed peritonitis and had to be euthanized at 3 days, 6 weeks, and 7 weeks, respectively. It appeared that all four dogs died of treatment-related toxicity, which was confirmed by necropsy results described under “Macroscopic Findings.”

Macroscopic Findings. A total of 11 dogs were treated via interstitial light delivery alone. Six of those dogs received only partial gland treatment (one light source placed in the left upper and right lower quadrants of each prostate) to evaluate lesion size. Groups of two dogs each received 6 mg/kg and 75 J/cm, 6 mg/kg and 150 J/cm, or 2 mg/kg and 100 J/cm. Dogs were euthanized 2 days after treatment. The lesions were well-circumscribed necrotic areas (Fig. 3) ranging in diameter from 12 to 15 mm around the axis of the cylindrical diffusing light source. There appeared to be no difference in diameter of necrosis among the various drug/light doses delivered.

Another five dogs were treated via interstitial light delivery alone with the intent of comprehensive coverage of the entire gland. The one dog receiving 6 mg/kg, 75 J/cm was treated at four sites and was euthanized at 2 weeks. Residual lesions were noted with areas of resolving necrosis throughout the gland. The remaining four dogs received 2 mg/kg, 100 J/cm. One dog was euthanized at 2 days, one at 2 weeks, and two at 3 months. At 2 days, necrosis was found throughout the entire gland (Fig. 4A). No obvious skip areas were noted in the dogs with smaller prostates. Dogs with larger prostates did have microscopic areas of unaffected tissue in “watershed” areas between light fiber sites. At 2 weeks, the lesions appeared to be resolving (Fig. 4B); and by 3 months, again the tissue appeared pale, but grossly normal (Fig. 4C).

A total of 11 dogs were treated by a combination of an interstitial and transurethral approach. Two of those dogs served as controls and were treated with light only (100 or 150 J/cm). Neither dog had visible lesions on gross sectioning. Another seven dogs received 6 mg/kg and 150 J/cm. Three of the seven were euthanized at 2 days. Each was treated with four to six interstitial sites and showed coalescence of necrotic lesions. No skip areas were noted (Fig. 5). The remaining four dogs treated with 6 mg/kg and 150 J/cm were scheduled to be euthanized at 3 months, but due to complications (as stated previously) they either died or were euthanized at 3 days, 3 weeks, 6 weeks, or 7 weeks after treatment. These specimens revealed complete destruction of urethral and central prostatic tissue with large fistulac between the urethra/prostate and peritoneum. Only an outer rim of prostate tissue could be identified grossly (Fig. 6). Finally, two dogs received 2 mg/kg, 100 J/cm and were euthanized at 3 months. These specimens showed intact urethral and prostate tissue, no residual necrosis, and pale-appearing tissue throughout the gland.

Five dogs were treated with transurethral light delivery alone. The dogs receiving 6 mg/kg, 75 J/cm and 6 mg/kg, 150 J/cm were euthanized at 1 and 2 weeks, respectively. The remaining three dogs received 2 mg/kg, 100 J/cm and were euthanized at 2 days, 2 weeks, and 3 months after treatment. Irrespective of drug and light dose, all dogs acutely showed zones of periurethral necrosis similar in size to those seen in the dogs treated via the interstitial alone approach. Healing of these lesions was again seen by 2 weeks with near complete resolution by 3 months.

Twenty dogs also underwent temperature measurements within the prostate tissue. At ~5 mm from the light source, the temperature increased from 0.5°C to 3.1°C from initiation to completion of light administration.

Microscopic Findings. No microscopic abnormalities were noted in either of the light only control dogs that were euthanized at 2 weeks and 3 months. In the treated dogs, at 2 days there was extensive hemorrhagic necrosis of the glandular tissue in the treatment areas (Fig. 7A) with marked local hemorrhagic vasculitis. The size of the induced lesions was similar regardless of dose of light or drug as was noted in the macroscopic findings. The fibromuscular connective tissue appeared to be affected in the same manner as the glandular tissue, showing diffuse and extensive necrosis. The prostatic capsule was also affected, showing a local inflammatory reaction. Treatment via the transurethral approach resulted acutely in disruption of the urethral epithelium (Fig. 7B).

At 2 weeks, the areas treated interstitially showed resolving necrosis with atrophic glandular epithelium (Fig. 8A). The areas treated transurethrally showed complete regeneration of the urethral epithelium with periurethral glandular atrophy (Fig. 8B).

At 3 months, the areas treated interstitially showed resolution of the necrosis with persistent glandular atrophy and squamous metaplasia (Fig. 9). The urethral epithelium was completely normal in all dogs with some persistent periurethral glandular atrophy. Fibrosis and loss of stromal connective tissue were noted throughout the prostate. Areas of chronic inflammation were noted throughout the gland. The prostatic capsule remained intact but also showed evidence of a low grade chronic inflammatory reaction.

The bladder neck appeared to be affected most markedly in the dogs receiving transurethral treatments. At 2 days, erythema and submucosal hemorrhage was noted in most of those dogs. Minimal erythema was noted in only one of the dogs treated interstitially alone. At 3 months, those dogs treated both tran-
surethrally and interstitially exhibited smooth muscle hypertrophy of the bladder neck. No abnormalities were seen in the dogs treated via either the interstitial or transurethral route alone.

The rectum appeared to be affected in four dogs, all of which received interstitial treatment. Three dogs had been euthanized at 2 days and showed focal areas of hemorrhage extending into the muscle layer which appeared to correspond with the position of the needles containing the diffusers. One dog was euthanized at 3 months and showed focal scarring into the muscle layer of the rectum, but no evidence of fistula formation.

Finally, local temperature elevations of 0.5°C-3.1°C caused by heat generated by the diffuser tips produced no observable tissue damage in the control dogs (who received light but no Lu-Tex) as analyzed pathologically 2 weeks and 3 months after treatment.

DISCUSSION

Photodynamic therapy in solid organs has considerable potential for treating a wide variety of cancers. The prostate is a good target organ for PDT because cancers are often locally confined and techniques already exist for the interstitial administration of radiation which could easily be adapted. PDT could

Fig. 4  A, 2 days after interstitial treatment of the entire prostate; B, 2 weeks after interstitial treatment of the entire prostate; C, 3 months after interstitial treatment of the entire prostate.

Fig. 5  Two days after combined interstitial and transurethral treatment of the entire prostate (6 mg/kg Lu-Tex, 150 J/cm 732 nm light).
provide a second chance for cure in cases of locally recurrent prostate cancer after prior radiation therapy in which salvage options are limited (9). It could even be used as an alternative primary therapy that avoids the exposure issues of radiation therapy and the surgical risks inherent to radical prostatectomy. Prior reports demonstrated that PDT can produce localized
necrosis in a canine prostate model using photosensitizers such as Photofrin, mTHPC, tin(II)ethyletiopurpurin dichloride, and aluminum disulfonated phthalocyanine (10–13). We chose to investigate the effect of the second-generation photosensitizer, Lu-Tex, due to its longer wavelength of activation, thus providing potentially greater light penetration through tissue as well as its short (24 h) duration of skin photosensitivity.

Prostate cancer has a high propensity to be multifocal within the prostate (14); thus, the entire gland must be treated to eradicate the tumor. Therefore, the primary goal of this study was to establish a photosensitizer/light dose and light delivery system that would allow complete treatment of the entire gland while maintaining an acceptable level of toxicity.

**Treatment Parameters and Technique.** No prior studies have been reported in the literature using Lu-Tex for PDT of the *in situ* prostate. Therefore, the initial dose of Lu-Tex (6 mg/kg) and 732 nm light fluence (75 J/cm at 150 mW/cm) was chosen based on experience in a murine model as well as human trials involving a number of different tumor types (15–17).

We chose a light delivery system that could be easily adapted to instrumentation currently used in patients to deliver interstitial radioactive seeds (transperineally) to the human prostate. However, an open transabdominal approach was chosen in our dog model, rather than the transperineal approach used in humans, simply for better access to the canine prostate. Plastic catheters (17-gauge) with a sharp end and a metal trocar were used in the present study because these catheters can be used with existing human prostate brachytherapy templates and can be seen on transrectal ultrasound imaging for accurate placement via the transperineal approach. Once positioned correctly, the metal trocars can be replaced with the laser diffusers.

Studies to evaluate lesion size produced by a single fiber revealed circular areas of well-demarcated necrosis 12–15 mm in diameter. Lesions of similar size were found at both the high (6 mg/kg, 150 J/cm) and low (2 mg/kg, 100 J/cm) dose levels. Due to the fixation process, a tissue shrinkage factor of ~23% (19) should be accounted for when assessing the true diameter of necrosis in the prostate. Therefore, the diameter of necrosis

![Figure 8](image-url)
surrounding an interstitial fiber using Lu-Tex-mediated PDT in the in situ prostate is ∼15.6 to 19.5 mm. This diameter of necrosis is larger than that reported for Photofrin (10) and aluminum disulfonated phthalocyanine (12) and roughly equivalent to mTHPC (11). The needle spacing used in our experiments (10 mm) to treat the entire gland was more than sufficient to cause confluent necrosis based on these initial findings and likely resulted in light overlap in some areas of the prostate. It could be argued that the needles could have been spaced as much as 15 mm apart and still cause confluent necrosis, but it was decided to keep the distance at 10 mm to allow for any inaccuracies of needle placement. With this needle arrangement, we were able to achieve confluent necrosis of the prostate with either combined transurethral and interstitial light delivery or interstitial light delivery alone. It is clear that all of the doses of Lu-Tex and far red light used in this study led to tissue necrosis. However, the minimum or threshold dose of photosensitizer and light needed to cause tissue necrosis has not been defined.

However, combined transurethral and interstitial treatment likely resulted in significant areas of light overlap which may have led to the unacceptable toxicities (urethral disruption and fistula formation discussed below) at the highest dose levels used (6 mg/kg, 150 J/cm). This approach of combining transurethral and interstitial light delivery was abandoned in the later stages of the study.

In several of the dogs treated with interstitial light delivery alone, microscopic foci of unaffected glandular tissue were noted in “watershed” areas between fiber sites. Inadequately treated microscopic regions of the prostate are unacceptable when designing treatment in patients with prostate cancer because of the multifocality of the disease (14). The “skip” areas noted were likely the result of our inability to precisely position the plastic needles in parallel to each other. This problem can be avoided by careful positioning of needles within the gland with the aid of transrectal ultrasound guidance and ensuring that every point in the prostate is no more than ∼7.5 mm from a needle site.

Also, this study evaluated the effects of PDT in a canine model of normal prostate tissue. Unfortunately, no large animal model exists to study prostate cancer in situ. Certainly, the model used in the present study provides information regarding the normal tissue toxicities of Lu-Tex-mediated PDT. Unfortunately, it is not possible to determine whether treatment results obtained in this study are predictive of efficacy in prostate cancer. However, there is no reason to believe that prostate adenocarcinoma should be less sensitive to PDT than normal prostate epithelium. In fact, based on preclinical murine data showing that tumor:normal tissue ratios of Lu-Tex are as high as 8.5–10.6 (16), it is possible that prostate cancer cells might be more sensitive to the treatment effects of PDT than is normal tissue. One final consideration is that there are differences in the optical properties of prostate tissue between humans and dogs (20), which further complicates a prediction regarding the efficacy of Lu-Tex-mediated PDT in humans.

**Toxicity.** The evaluation of toxicity of Lu-Tex-mediated PDT in the canine prostate was made through clinical observation, endoscopy, and microscopic examination. Critical structures that were evaluated after PDT included the urethra, rectum, and prostatic capsule.

Urethral damage was evident in all dogs receiving treatment via the transurethral route. Those treated with a combination of transurethral and interstitial light delivery at the highest dose level (6 mg/kg, 150 J/cm) showed the greatest damage. Cystoscopic evaluations performed within days after PDT revealed severe erythema and necrosis within the urethral lumen that persisted for weeks afterward. Microscopically, the urethral epithelium was clearly disrupted with evidence of periurethral vascular damage. Four dogs treated at the highest dose level developed urethral-peritoneal fistulae and subsequently died or required euthanasia at 3 days or 3, 6, or 7 weeks after PDT. Urinary retention might have been expected given the great degree of edema and luminal necrosis, but the development of urethral fistulae may have masked this clinical problem before death. The two dogs receiving combined interstitial and transurethral treatment at the lower dose level (2 mg/kg, 100 J/cm), however, did not suffer any clinically apparent acute side effects, although one did develop a cystoscopically detected urethral stricture.

The dogs treated with transurethral light alone at all dose levels did not develop urinary incontinence but did have erythema and mild necrosis as demonstrated by cystoscopy shortly after PDT. The urethral epithelium was disrupted on microscopic evaluation of the tissues at 2 days. However, by 2 weeks, no abnormalities were evident on any evaluation. The dogs treated with interstitial light alone at all dose levels likewise did not develop urinary incontinence and, as one might expect, showed the least amount of urethral damage endoscopically and microscopically. Thus, significant urethral damage and the development of urinary incontinence appeared to be related to high doses of both light (likely due to overlap from interstitial and transurethral sources) and drug to the urethra and urethral sphincter, emphasizing the importance of minimizing the PDT dose to those critical structures. This phenomenon is well known in the practice of radioactive seed brachytherapy. Treatment planning techniques use a “peripheral loading” arrangement in which seeds are placed mainly in the periphery of the prostate (where most prostate tumors are found) such that the dose to the...
urethra is no more than 100% of the prescribed dose (21). Such a system for interstitial prostate PDT might also help to limit urethral toxicity.

Rectal damage was not apparent on clinical and endoscopic evaluations of all dogs. However, in the dogs receiving interstitial treatment, focal areas of hemorrhage and eventually scar were noted microscopically and appeared to correspond to needle tracks in which the needles were pushed too far through the prostate and into rectum. This was a consequence of the trans-abdominal technique used in this study and likely could be avoided with a transperineal approach with transrectal ultrasound guidance.

The prostatic capsule showed both acute and chronic diffuse capsular inflammation with percapsular vasculitis in all areas treated via an interstitial approach. This finding is contrary to that found by Chang et al. (11), who noted no connective tissue (including capsule) effect using mTHPC PDT in the canine prostate. This difference may be due to differences in connective tissue uptake of mTHPC and Lu-Tex, although it could also be related to the PDT doses that were delivered.

The results of this study demonstrated that Lu-Tex PDT is feasible in the canine prostate. Its biological effect is characterized initially by inflammation and necrosis followed by glandular atrophy and fibrosis. This tissue effect was similar to that of PDT mediated by other photosensitizers. In addition, this study showed that both the interstitial alone and the combined interstitial and transurethral technique of light delivery could provide comprehensive treatment of the entire prostate gland, whereas the interstitial alone technique resulted in the least amount of urethral damage. Although questions remain regarding the PDT effect on prostatic carcinoma, it seems reasonable based on our data to proceed with Phase I human trials in a selected population such as those patients with locally recurrent disease after radical radiotherapy.

REFERENCES
Photodynamic Therapy in the Canine Prostate Using Motexafin Lutetium

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