Preclinical Evaluation of Motexafin Lutetium-mediated Intraperitoneal Photodynamic Therapy in a Canine Model

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ABSTRACT

Intraperitoneal photodynamic therapy (IP PDT) is an experimental cancer treatment in clinical development for the treatment of peritoneal carcinomatosis and sarcomatosis. A canine study of motexafin lutetium (Lu-Tex)-mediated IP PDT was performed to evaluate normal tissue toxicities of this treatment in the presence and absence of a bowel resection and to assess the feasibility of measuring Lu-Tex fluorescence in abdominal tissues. Thirteen dogs were treated with Lu-Tex (0.2–2 mg/kg) i.v. 3 h before laparotomy and 730-nm light delivery (fluences, 0.5–2.0 J/cm^2; average fluence rate <150 mW/cm^2). Laparoscopy was performed 7–10 days after the procedure to assess acute toxicities. *In situ* fluorescence spectra were obtained from various abdominal tissues before and after light delivery using a fiber array probe with fixed-source detector distances. Lu-Tex-mediated IP PDT was well tolerated at the doses of drug and light studied. Bowel toxicity was not observed in animals treated with a bowel resection before PDT. Mild transient liver function test abnormalities without associated clinical sequelae were observed. No gross PDT-related abnormalities were observed at laparoscopy or necropsy; however, thickening in the glomerular capillary wall and the mesangium were noted microscopically in the kidneys of seven dogs. No renal function abnormalities were found. Analysis of the fluorescence spectra from intra-abdominal tissues suggests that measurements of Lu-Tex *in situ* are feasible and may provide a way of assessing photosensitizer concentration *in vivo* without the need for a biopsy. These results support the continued development of Lu-Tex as a candidate photosensitizer for IP PDT.

INTRODUCTION

The spread of carcinomas and sarcomas to the peritoneal surfaces, known as carcinomatosis or sarcomatosis, is a common clinical event in some cancers (1). This pattern of cancer spread is usually incurable with standard therapies (2–4). PDT[2] is a superficial cancer treatment that requires a photosensitizer, light, and oxygen for cytotoxicity (5). IP PDT is currently being evaluated in patients for the treatment of carcinomatosis and sarcomatosis (5–7). PDT is a potentially ideal treatment for peritoneal surface malignancies because the cytotoxic treatment effects are superficial and thus spare underlying tissues (8). Studies of IP PDT in rodent models of carcinomatosis have demonstrated the potential efficacy of this treatment approach (9–14). A human Phase I study of IP PDT with the first generation photosensitizer, Photofrin, for disseminated i.p. malignancies has established the maximally tolerated dose of this photosensitizer and light (6, 7). A Phase II clinical trial of Photofrin-mediated IP PDT is in progress to determine the efficacy of this treatment in defined patient populations with recurrent carcinomatosis and sarcomatosis (5, 15).

The preliminary results of the Phase II trial of IP PDT suggest that this treatment approach is feasible but not uniformly successful (15). In addition, some toxicities have been observed, including postoperative fluid shifts, hypotension, hydronephrosis, pleural effusions, enteric fistula, transient LFT abnormalities, thrombocytopenia, and wound dehiscence (15). It is clear that optimization of IP PDT is required. One potential avenue for optimizing IP PDT is the development of second generation photosensitizer-light combinations. The second generation photosensitizer motexafin lutetium [Lu-Tex (lutetium texaphyrin)] is a pentadentate aromatic metallotexaphyrin with an absorption band at 732 nm. Lu-Tex-mediated PDT has reported efficacy in several murine tumor models (16–18). Promising results from Phase I and II clinical trials of Lu-Tex-mediated PDT have been reported in patients with cutaneous malignancies and recurrent breast cancer (19, 20).

The potential advantages of Lu-Tex as a photosensitizer are as follows: (*a*) it is a pure, water soluble compound; (*b*) selectivity of photosensitizer retention in tumor compared with normal tissues has been observed in murine tumor models (16); and (*c*) clinical use is associated with only 24–48 h of skin photo-

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[2] PDT, photodynamic therapy; IP PDT, i.p. PDT; ALT, alanine transferase; AST, aspartate transferase; AlkP, alkaline phosphatase; LFT, liver function test.

Table 1 Dose-escalation schema and acute clinical effects for all dogs treated with Lu-Tex IP PDT[a]

Dog no.	Drug dose (mg/kg)	Light dose (J/cm2)	Bowel resection	Wound complications	Acute clinical effects	Late clinical effects
1	0	0.5	No	None	None	None
2	0.2	0.5	No	None	Vomited once	None
3	0.2	0.5	No	None	None	None
4	2	0.5	No	Seroma	None	NA[b]
5	2	0.5	Yes	None	None	None
6	2	0.5	No	None	None	None
7	2	0.5	No	Seroma	None	None
8	2	0.5	Yes	None	Vomited once	None
9	0	2.0	No	None	None	None
10	2	2.0	No	Seroma	Vomited once	None
11	2	2.0	Yes	None	None	None
12	2	2.0	No	None	None	None
13	2	2.0	Yes	None	Vomited once	None

[a] No major acute or late clinical effects were found.
[b] Dog died during the follow-up laparoscopy.

sensitivity (19). Furthermore, because Lu-Tex is activated by the near-infared wavelength, 730 nm, there is likely to be less interference by blood during light delivery compared with the shorter wavelengths of light used to activate other photosensitizers. One potential disadvantage of Lu-Tex for use in IP PDT is that activation by 730 nm light may lead to a treatment effect that extends deeper into the abdominal tissues compared with the light used to activate other photosensitizers. This has the potential to cause greater normal-tissue toxicity compared with photosensitizers activated by shorter-wavelength light. We performed a normal tissue tolerance study of Lu-Tex-mediated IP PDT to investigate the toxicities of Lu-Tex-mediated PDT. A canine model was chosen for this study because of previous work demonstrating the utility of this model in the preclinical evaluation of Photofrin-mediated IP PDT (21). The objectives of the present study were: (*a*) to evaluate the toxicities of Lu-Tex-mediated IP PDT in a canine model using doses of photosensitizer (19) and light (6) similar to those used in previous human clinical trials; (*b*) to determine the toxicity of IP PDT in the presence of a bowel resection; and (*c*) to test the feasibility of measuring Lu-Tex-reflective fluorescence in canine abdominal tissues before and after PDT *in vivo*.

MATERIALS AND METHODS

Motexafin Lutetium. Lu-Tex was supplied as a 2 mg/ml aqueous solution by Pharmacyclics, Inc. (Sunnyvale, CA). The compound was stored in a refrigerator and protected from light. The Lu-Tex solution was drawn into a syringe and injected undiluted into the cephalic vein of the dogs over a period of 5–10 min via a 20-gauge i.v. catheter. The volume administered to each animal was calculated from the weight of each dog and the dose level in mg/kg.

Experimental Animals. Thirteen dogs (10 laboratory Beagles and 3 mixed-breed dogs) ranging in age from 8–17 months (one dog was of unknown age) and weighing 9.0–23.6 kg were used in this study. The animals were housed in indoor runs (22) and fed Purina lab canine chow (Ralston Purina Company, St. Louis, MO) and water *ad libitum*. All animals had been fully vaccinated, treated for intestinal parasites and quarantined for 2 weeks before starting the study. One dog tested positive for a *Giardia* spp. This animal and four others in contact with this dog were treated with metronidazole 30 mg/kg p.o. once daily for 5 days as per routine clinical care. Each animal had a complete blood count and serum chemistries (electrolytes, blood urea nitrogen, creatinine, glucose, and LFTs) drawn before treatment. All baseline values were within normal ranges except for one dog with a mild (grade I; see definitions below) elevation of ALT. Animals were cared for under the supervision of veterinarians from the University of Pennsylvania School of Veterinary Medicine (Philadelphia, PA). The experimental protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

i.p. PDT. Lu-Tex was administered i.v. 3 h before planned light delivery. This drug-light interval was chosen because, in preclinical models, 3 h was associated with maximal efficacy (16, 17), and because 3–5 h was used initially for the human clinical trial (19). The initial photosensitizer dose was 0.2 mg/kg, which was escalated to 2 mg/kg. The dogs subsequently underwent a laparotomy with exposure of the entire abdominal contents. Light was delivered to 13 dogs after exposure of the abdominal contents. A small-bowel resection was performed before light delivery in four dogs. The treatment parameters for each dog are described below and presented in Table 1.

Laser light was generated with a Diomed diode laser (Diomed, Cambridge, England) and was provided by Pharmacyclics, Inc. (Sunnyvale, CA). The maximum power output of the laser was 2 W. Two dogs served as light-only controls, receiving 730 nm light (dog 1 at 0.5 J/cm^2 and dog 9 at 2 J/cm^2) but no photosensitizer. Two dogs (dogs 2 and 3) were treated with lutetium texaphyrin, 0.2 mg/kg, and 730 nm light at 0.5 J/cm^2. Five dogs (dogs 4–8) were treated with lutetium texaphyrin, 2 mg/kg, and 730 nm light at 0.5 J/cm^2. Two of these five dogs (dog 5 and 8) underwent bowel resections. Four dogs (dogs 10–13) were treated with lutetium texaphyrin, 2 mg/kg, and 730 nm light at 2.0 J/cm^2. Two of these four dogs (dogs 11 and 13) underwent bowel resections. For all light treatments, the average fluence rate was $\leq$150 mW/cm^2. No protective filters were placed on the operating room lights, although the lights were dimmed during light delivery.

After opening the abdominal cavity, spherical light detectors (Rare Earth Medical, West Yarmouth, MA) were placed for

monitoring of the light fluence rate and the cumulative light fluence. Using silk sutures, the detectors were placed in the right upper quadrant, the left upper quadrant, the right peritoneal gutter, the left peritoneal gutter, and in the midline anterior pelvis as described previously for human IP PDT (6). Two mobile spherical detectors were also used to measure light fluence at sites where fixed detectors could not be placed. The isotropic light detectors measured both incident and scattered light with an accuracy of ±15%. The detectors were connected to photodiodes (Photop UDT-455; Graseby Electronics, Orlando, FL), the output of which was converted, displayed, and stored on a personal computer. The light dosimetry system was developed in the Department of Radiation Oncology at the University of Pennsylvania and is based, in large part, upon a system described previously (23, 24). Detectors were calibrated in air in an integrating sphere with a diffuse light field as described previously (24). The detectors were placed in sterile i.v. tubing and filled with saline or air to match the refractive index of the surrounding medium before being placed in the dogs. In all dogs, delivery of the prescribed light fluence to each site within the abdomen was documented with this light dosimetry system.

The peritoneal surfaces were illuminated with 730 nm light using the same technique used in human clinical trials of IP PDT (6, 15, 25). The total light fluence ranged from 0.5–2.0 J/cm^2 (Table 1). The surfaces of the mesentery, small bowel, and large bowel were treated first with a flat-cut optical fiber emitting a circular beam of light. The tissues were flattened out on wet towels and illuminated in segments.

After delivery of light to the mesentery and bowel, the abdomen was filled with a dilute solution of intralipid (0.01%) in Lactated Ringers supplemented with magnesium and calcium. The remainder of the peritoneal surfaces, including the surfaces of the right upper quadrant, left upper quadrant, liver, spleen, omental bursa, right peritoneal gutter, left peritoneal gutter, and pelvis, were illuminated with 730 nm light using an optical fiber sheathed within a modified endotracheal tube (25). The balloon cuff of the endotracheal tube was inflated and filled with 0.1% intralipid. This light delivery system was designed to enhance light diffusion and minimize thermal effects from the light.

Eye protection in the form of goggles was made available to all operating room personnel. After the administration of light, the spherical detectors were removed from the abdomen and passed from the surgical table before abdominal closure.

Surgical Procedure. Before surgery, the dogs were not permitted to eat for 12 h. All medications were purchased from the University of Pennsylvania School of Veterinary Medicine Pharmacy. Premedication consisted of 0.1 mg/kg acepromazine maleate (Fermenta Animal Health Co., Kansas City, MO), 0.02 mg/kg atropine sulfate (Phoenix Scientific, Inc., St. Joseph, MO), and 0.5 mg/kg morphine sulfate (Astra Pharmaceutical Products, Inc., Westborough, MA) by i.m. injection given 20 min before induction of general anesthesia. The dogs were anesthetized with 10 mg/kg thiopental sodium (Abbott Laboratories, North Chicago, IL) i.v. and intubated with an endotracheal tube (Rusch, Duluth, GA). General anesthesia was maintained with an inhaled mixture of oxygen and isoflurane. During surgery, animals received a constant i.v. infusion of Normosol-R (Abbott Laboratories, North Chicago, IL) at a rate of 20 ml/kg/h. Cefazolin sodium, 20 mg/kg (Marsam Pharmaceuticals, Inc., Cherry Hill, NJ) was administered i.v. every 2 h during the surgical procedure. While under anesthesia, ECG tracings, blood pressure, and oxygen saturation were monitored continuously.

After the induction of anesthesia, the animals were placed in a supine position with the caudal aspect of the animal slightly elevated. Hair was clipped from the ventral abdomen with a no. 40 clipper blade (Oster, Sunbeam, Inc., Boca Raton, FL) and prepared with a dilute povidone-iodine scrub solution.

The surgical field was four-quarter draped, and a ventral midline incision was made from the xyphoid to the pubis. The abdominal organs were examined for any abnormalities. In the four animals that had bowel resections before undergoing PDT, a 4-cm section of jejunum was resected and an end-to-end anastomosis was performed using a single layer of simple interrupted sutures with 3–0 polydioxanone (PDS II; Ethicon, Inc., Cincinnati, OH). 730 nm light was then delivered as described above. Once the light therapy was completed, the abdomen was closed in 3 layers using polydioxanone sutures for the abdominal wall and s.c. tissues and monofilament nylon (Ethilon; Ethicon, Inc., Cincinnati, OH) for the skin. The dogs were monitored closely until they recovered from anesthesia. Butorphanol tartarate (Torbugesic; Fort Dodge Animal Health, Fort Dodge, IA), 0.5 mg/kg i.m., was given as needed for postoperative pain.

The treated dogs were examined twice daily for the first 5 days post-PDT and 5 times weekly after that. Blood tests were drawn twice in the first postoperative week and as clinically indicated thereafter. The following blood tests were obtained: (*a*) a complete blood count, including hemoglobin, hematocrit, WBC count including differential count, and an estimate of platelet numbers; (*b*) a chemistry panel including glucose, urea nitrogen (BUN), creatinine, total protein, albumin, globulin, total bilirubin, AlkP, AST and ALT, cholesterol, total calcium, phosphorus, sodium, potassium, and chloride. LFT abnormalities that were detected postoperatively were graded as follows: grade I , ≤2.5 × normal; grade II <2.5–5 × normal; grade III >5 × normal. LFTs were obtained within the first 5 days after surgery and then repeated at least one additional time thereafter.

Laparoscopy. Seven to 10 days after PDT, all dogs underwent an abdominal laparoscopy with biopsies, done with a 16-gauge tru-cut needle, obtained from the left lateral or left medial lobe of the liver, the peritoneum from the left lateral body wall ventral to the left kidney, and the skin on the anterior abdominal wall within the draped area of the surgical field but distant from the laparotomy incision. The dogs were not permitted to eat for 12 h before the procedure. Premedications and general anesthesia were delivered as described above. Cefazolin and Normosol-R were also given as described above. Once anesthetized, animals were placed in a supine position and prepared for surgery using a dilute chlorhexidine scrub solution.

Carbon dioxide pneumoperitoneum was established using a technique described previously (26). The intra-abdominal pressure was kept between 10–15 mm Hg during the procedure. A 10-mm camera port was placed just below the umbilicus, and two 5-mm operating ports were placed in the left and right paracostal areas. The abdomen was explored for evidence of gross lesions associated with PDT. Biopsies were taken of the

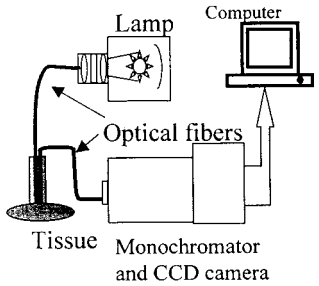

Fig. 1 The experimental set-up for measuring *in situ* fluorescence spectra consists of a CCD camera, a monochromator, and two optical fibers.

liver using a 14-gauge biopsy needle (Precisioncut; Becton Dickinson, Rutherford, NJ). Biopsies were also taken of the peritoneum and skin. All biopsy samples were placed in 10% buffered formalin and processed for pathological evaluation as described below. The laparoscopy incisions were closed using PDS and monofilament nylon (Ethicon, Cincinnati, OH). Skin sutures were removed 14 days after surgery. Butorphanol tartarate was administered, 0.5 mg/kg i.m., as needed for postoperative discomfort. Animals were monitored closely until fully recovered. The laparoscopic equipment was provided by Karl Storz Veterinary Endoscopy-America, Inc. (Goleta, CA) and Kendall Company (Bethel, CT). One dog (dog 4; Lu-Tex 2 mg/kg; light, 0.5 J/cm^2) suffered a cardiorespiratory arrest during the procedure. This animal's death was attributed to anesthesia, and a necropsy revealed no evidence of a laparoscopic or PDT-related cause of death.

The dogs were monitored for 60 days post-PDT and were then killed using 190 mg/kg pentobarbital sodium (Fatal-plus; Vortech Pharmaceuticals, Dearborn, MI) by i.v. injection. All animals underwent a full necropsy to evaluate for the presence PDT-related tissue damage. Samples were taken of skin, peritoneum, liver, kidney, and large and small intestine and were placed in 10% buffered formalin.

Preparation of Pathology Specimens. The tissue samples obtained at laparoscopy and necropsy were fixed and stored in 10% buffered formalin until processing. Core biopsies and trimmed tissues from necropsy (small intestine, large intestine, kidney, liver, skin, and peritoneum) were embedded in paraffin, sectioned, and stained with H&E by Idexx Veterinary Service, Inc. (West Sacramento, CA). Intestinal samples were cut such that sections contained full-thickness intestinal wall. Skin and peritoneum samples were also sectioned to contain the full thickness of tissue.

Review of Pathology Specimens. All specimens were examined with light microscopy by a board-certified veterinary pathologist (F. D. P.), who was blinded to the treatment groups. The density of tissue fibrosis was examined using a polarizer.

***In situ* Fluorescence Measurements.** *In situ* optical measurements of abdominal tissues were made to evaluate the feasibility of detecting Lu-Tex fluorescence. The experimental set up for measuring *in situ* fluorescence spectra consists of three major parts: a charge-coupled device camera, a monochromator, and two optical fibers (Fig. 1).

Two 640-μm diameter optical fibers were used: one for excitation and one for the collection of the fluorescence signal. A tungsten-halogen 200-W (Oriel Instruments, Stratford CT) with a 488-nm interference filter (Edmunds, Barrington, NJ) and a condenser were used to excite the sample emission (~1 μW fiber output at the tissue). The scattered fluorescence signal was collected by the detection fiber positioned 1.3 mm away from the source. The fluorescence signal was directed to a charge-coupled device camera (Acton Research, Acton, MA) and transferred to a personal computer.

Measurements of Lu-Tex fluorescence spectra were first made in a 10% intralipid phantom solution (Fresenius Kabi Clayton L.P., West Clayton, NC). Increasing concentrations of Lu-Tex were added to the phantom, and fluorescence spectra were collected to evaluate linearity of the system response. Data from an analysis of the area-under-the-curve of the fluorescence peak were proportional to Lu-Tex concentration up to 10 mg/ml (data not shown). After the ventral midline incision was made in the dogs, the source and detection fibers were placed on the surface of abdominal organs (peritoneum, liver, kidney, and bowel) under sterile conditions. One measurement was taken on each organ before the initiation of light delivery (but after Lu-Tex delivery) and after the completion of light delivery. After completion of the optical fluorescence measurements, the fibers were removed from the surgical field. Measurement times were typically 10 s.

RESULTS

Clinical Findings. All of the dogs tolerated IP PDT without major acute (Table 1) or late clinical effects except those associated with prolonged general anesthesia and a laparotomy. One dog died during the follow-up laparoscopy procedure. This death was thought to be anesthetic-related, and at necropsy no PDT-related tissue damage was found. The postoperative course was similar for both control (light only) and PDT-treated animals. All animals were eating normally within 2 days of treatment. Ten of 12 animals gained weight during the postoperative course. Four dogs vomited on one occasion on the first postoperative day. The vomiting was not clearly related to Lu-Tex or light delivery. Three dogs developed seromas at the site of their abdominal incisions, the result of these animals chewing their incisions postoperatively. In all cases, the seromas were drained at the time of laparoscopy. One seroma became infected and the animal was treated successfully for 10 days with amoxicillin-clavulanate (Clavamox; Pfizer Animal Health, Exton, PA). All seromas resolved after local care was instituted and did not recur. No skin photosensitivity was observed.

All treated dogs and one control dog showed transient elevations in the LFTs, AlkP, AST, and ALT (Table 2). Most LFT abnormalities were detected in the first 5 days after surgery and IP PDT. All values returned to normal within 4–10 days posttreatment. The LFT abnormalities were not associated with clinical sequelae. The majority of the LFT elevations were mild (grades I or II), although one dog was noted to have a transient grade III elevation of ALT and AST, and one dog had a transient grade III elevation of AST. The severity of LFT abnormalities was not clearly correlated to the dose levels of Lu-Tex or light. An elevated neutrophil count was present after treatment in 8 of 13 dogs, including both control dogs. The WBC returned to

Table 2 Post-PDT laboratory findings

Transient elevations in liver function tests and white blood cell counts were found after surgery and IP PDT. The laboratory abnormalities were detected in both treated and control dogs. These were not clearly PDT-related. AlkP-alkaline phosphatase, AST-aspartate transferase, ALT-alanine transferase, WBC-white blood count. Grade I <2.5 × normal; grade II 2.5–5 × normal; grade III >5 × normal.

Dog no.	Drug dose (mg/kg)	Light dose (J/cm2)	Bowel resection	AlkP	AST	ALT	WBC
1	0	0.5	No	Normal	Grade I	Grade I	Increased
2	0.2	0.5	No	Grade II	Grade II	Grade I	Increased
3	0.2	0.5	No	Grade I	Grade II	Grade I	Normal
4	2	0.5	No	Normal	Grade II	Grade I	Increased
5	2	0.5	Yes	Grade I	Grade III	Grade III	Normal
6	2	0.5	No	Normal	Grade I	Grade I	Normal
7	2	0.5	No	Normal	Grade II	Grade I	Increased
8	2	0.5	Yes	Grade I	Grade I	Grade I	Normal
9	0	2.0	No	Normal	Normal	Normal	Increased
10	2	2.0	No	Grade I	Grade I	Grade I	Increased
11	2	2.0	Yes	Normal	Normal	Normal	Normal
12	2	2.0	No	Grade I	Grade II	Grade I	Increased
13	2	2.0	Yes	Grade I	Grade III	Grade II	Increased

Table 3 Laparoscopic biopsy findings

Mild to moderate fibrosis was observed in treated and control dogs, mainly at previous biopsy sites. Mild or moderate hepatic vacuolization was detected in the biopsies of six dogs, including one control dog. No other microscopic abnormalities were detected.

Dog no.	Drug dose (mg/kg)	Light dose (J/cm2)	Bowel resection	Skin	Liver	Peritoneum
1	0	0.5	No	NSL[a]	Mild hepatocellular vacuolization	Moderate fibrosis
2	0.2	0.5	No	NSL	NSL	NSL
3	0.2	0.5	No	NSL	NSL	NSL
4	2	0.5	No	NSL	NSL	Moderate fibroplasias
5	2	0.5	Yes	NSL	Moderate hepatocellular vacuolization	NSL
6	2	0.5	No	NSL	Mild hepatocellular swelling	Moderate fibrosis
7	2	0.5	No	NSL	Moderate hepatocellular vacuolization	Moderate fibrosis
8	2	0.5	Yes	NSL	Moderate hepatocellular vacuolization	Moderate fibrosis
9	0	2.0	No	NSL	NSL	Mild fibrosis
10	2	2.0	No	NSL	Moderate hepatocellular vacuolization	Moderate fibrosis
11	2	2.0	Yes	NSL	NSL	NSL
12	2	2.0	No	NSL	NSL	NSL
13	2	2.0	Yes	NSL	NSL	NSL

[a] NSL, no significant lesions.

normal within 2–4 days in all cases except the one dog with an infected seroma. Three dogs had a mild transient decrease in albumin. No other hematological or biochemical abnormalities were noted.

Laparoscopic Findings. On laparoscopic examination, all dogs were found to have adhesions. These were located on the omentum, ventral midline, liver lobes, and bowel resection sites and were likely related to the biopsy or resection procedures performed at the time of laparotomy 7–10 days earlier. There were no gross differences between the control and the IP PDT-treated dogs. Examination of the liver, spleen, kidneys, pancreas and bowel revealed no significant abnormalities. No dog had evidence of ascites, peritonitis, or bowel perforation.

Necropsy Findings. At necropsy, 60 days after IP PDT, all dogs had adhesions in similar locations as described during the laparoscopy. There were no gross differences between the control and the IP PDT-treated dogs. No pleural effusions or ascites were noted. All intestinal anastomoses were patent and had healed normally. There were no bowel perforations. There were occasional fibrin deposits on the liver surface. All other organs were grossly normal.

Pathology Review. In general, lesions detected in biopsies taken at the time of the laparoscopy were mild and considered nonsignificant (Table 3). Biopsies of skin taken at laparoscopy revealed no significant lesions in any dog. Peritoneal biopsies showed no significant lesions in six dogs, mild fibrosis in one dog, and moderate fibrosis in six dogs. Both control dogs showed fibrosis (one mild and one moderate). Liver biopsies were normal in seven dogs, including one control. The remainder of the liver biopsies demonstrated mild hepatocellular swelling (one dog), mild hepatocellular vacuolization (one control dog), and moderate hepatocellular vacuolization (four dogs). No other microscopic abnormalities were noted from the laparoscopic biopsies.

In tissue samples taken at necropsy (Table 4), the gross findings of peritoneal fibrosis described above were not identified. All dogs, including control dogs, had a mild-to-moderate plasmacytic enteritis (five, mild; eight, moderate). In five animals, including both control dogs, there was a plasmacytic colitis (four, mild; one, moderate). Thickening of the mesangium and glomerular capillary wall within the kidneys was noted in seven dogs, (five, mild; two, moderate). No kidney

Table 4 Necropsy results

A mild plasmacytic enteritis was observed in control as well as treated dogs. Mild mesangial thickening of the kidneys of unclear significance was observed in dogs receiving IP PDT.

Dog no.	Drug (mg/kg)	Light (J/cm2)	Bowel resection	Skin	Liver	Peritoneum	Kidney	Small intestine	Large intestine
1	0	0.5	No	NSL[a]	NSL	NSL	NSL	Mild enteritis	Mild enteritis
2	0.2	0.5	No	NSL	NSL	NSL	NSL	Mod[b] enteritis	NSL
3	0.2	0.5	No	NSL	NSL	NSL	Mild MT[c]	Mod enteritis	Mild enteritis
4	2	0.5	No	NSL	NSL	NSL	NSL	Mild enteritis	NSL
5	2	0.5	Yes	NSL	NSL	NSL	NSL	Mild enteritis	Mild enteritis
6	2	0.5	No	NSL	NSL	NSL	Mod[d] MT	Mod enteritis	NSL
7	2	0.5	No	NSL	Subcapsular fibrosis	NSL	Mod MT	Mod enteritis	NSL
8	2	0.5	Yes	NSL	NSL	NSL	Mild MT	Mild enteritis	NSL
9	0	2.0	No	NSL	NSL	NSL	NSL	Mod enteritis	Mild enteritis
10	2	2.0	No	NSL	NSL	NSL	Mild MT	Mod enteritis	NSL
11	2	2.0	Yes	NSL	NSL	NSL	Mild MT	Mod enteritis	Mild enteritis
12	2	2.0	No	NSL	Hepatocellular swelling	NSL	Mild MT	Mod enteritis	NSL
13	2	2.0	Yes	NSL	NSL	NSL	NSL	Mod enteritis	Mod enteritis Focal severe fibrosis

[a] NSL, No significant lesions.
[b] Mod, moderate.
[c] MT, mesangial thickening.

abnormalities were noted in either control dog. The significance of these lesions is unknown. No animal showed any clinical or biochemical evidence of impaired renal function. In 11 dogs, including both controls, there were no histological abnormalities found in the liver. Hepatocellular swelling was found in one dog, and subcapsular fibrosis was found in another.

***In situ* Fluorescence Measurements.** Fluorescence spectra (Fig. 2) were first made of Lu-Tex added to an intralipid phantom. Increasing concentrations of Lu-Tex (0–15.7 mg/ml) were added to the intralipid. The fluorescence spectra recorded from the phantom (Fig. 2*A*) demonstrate an increasing peak height in the region of 730–750 nm consistent with an increase in the Lu-Tex concentration. The shape of the fluorescence spectrum is consistent with the spectra previously reported for Lu-Tex (16). Fluorescence reflectance spectra of bowel in a control dog that had not received photosensitizer showed no fluorescence peak in the 730–750-nm range (Fig. 2*B*). In a dog that received 2 mg/kg Lu-Tex, spectra taken before and after light delivery are shown (Fig. 2*B*). The spectra obtained before light delivery were qualitatively similar to those detected in the phantom. A decrease in the fluorescence peak was observed after light delivery.

DISCUSSION

IP PDT with the first generation photosensitizer, Photofrin, is currently being evaluated in a Phase II trial of patients with i.p. carcinomatosis and sarcomatosis. Although some efficacy was reported in the Phase I trial of IP PDT, substantial toxicity has been associated with the treatment (6). The dose-limiting toxicity of Photofrin-mediated IP PDT in humans is bowel damage manifested by fistulae or perforations (6). Preclinical studies of tetra(*m*-hydroxyphenyl)chlorin-mediated IP PDT have also demonstrated that bowel toxicity is a major toxicity (27). One potential way to improve IP PDT is to use a photosensitizer such as Lu-Tex that has greater selective retention within tumor tissue compared with normal tissues. Because the cytotoxicity of PDT is dependent upon the simultaneous presence of both photosensitizer and light within tissues, such selective retention could improve the therapeutic gain of this treatment. Photosensitizer selectivity for tumor would potentially permit the use of light doses adequate to treat tumor but inadequate to damage normal tissues.

The primary objective of this study was to evaluate the normal tissue effects of Lu-Tex-mediated IP PDT. The technique used to deliver IP PDT in this study was the same as that used for human clinical protocols (6, 15, 25). The doses of photosensitizer and light in this study were not escalated to the point where unacceptable toxicity was observed in normal tissue. Instead, we chose to evaluate a dose of Lu-Tex and 730 nm light that is likely to be clinically relevant in a human clinical trial. Lu-Tex, in this study, was escalated to a dose (2 mg/kg) that has been used clinically (20). Doses of light were escalated to a fluence (2.0 J/cm^2) near the maximally tolerated dose for bowel in patients who have been treated with Photofrin-mediated IP PDT (7). The highest fluence used in the present study was higher than the doses used during the preclinical investigation of Photofrin-mediated IP PDT (21).

The results of this study demonstrate that Lu-Tex-mediated IP PDT using a photosensitizer dose of 2 mg/kg administered 3 h before 730 nm light delivery with a fluence of 2 J/cm^2 is well tolerated in a canine model. No substantial clinical toxicities beyond those routinely encountered after a laparotomy were observed. Importantly, no skin photosensitivity, bowel damage, or other clinically significant organ toxicity was noted. A laparoscopy was performed to directly visualize the abdominal contents and the peritoneal surfaces 7–10 days after IP PDT. No visible acute toxicities were noted on laparoscopic evaluation other than the formation of adhesions at previous biopsy sites. Likewise no significant gross abnormalities were found at necropsy 60 days after treatment.

A second objective of this study was to determine the toxicity of combining a bowel resection and anastamosis with IP PDT. Bowel resections are common during the debulking surgery re-

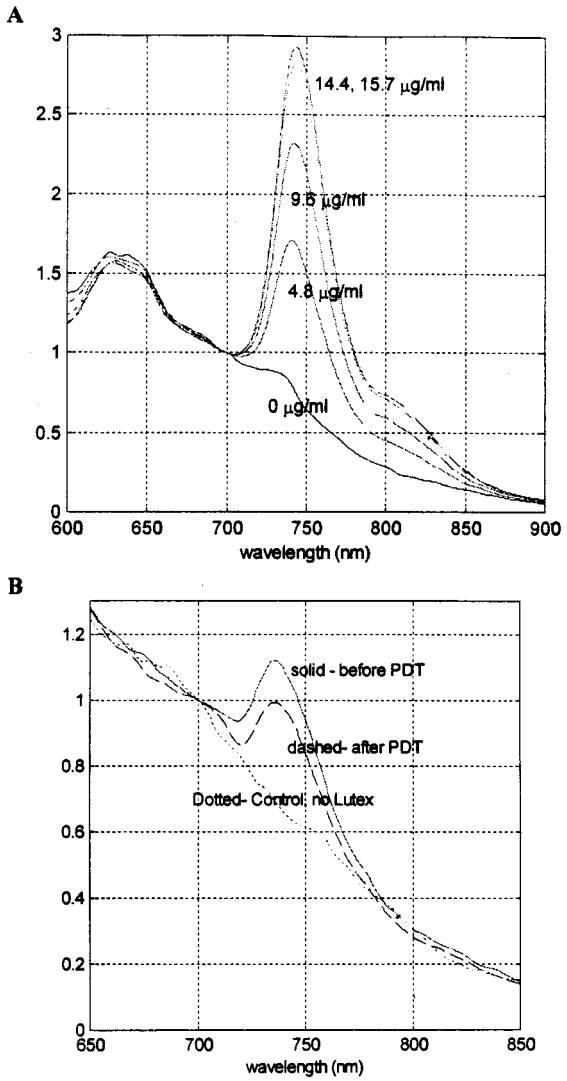

Fig. 2 *In situ* fluorescence measurements. *A,* fluorescent spectra taken of increasing Lu-Tex concentrations in a 10% intralipid phantom. Concentrations of Lu-Tex were: no Lu-Tex; 4.8 μg/ml; 9.6 μg/ml; 14.4 μg/ml; and 15.7 μg/ml. An emission peak is observed in the 730–750-nm range consistent with known Lu-Tex fluorescence spectra (16). An increase in the fluorescence peak is observed with increasing concentrations of Lu-Tex. *B,* fluorescence spectra of the canine bowel. *Dotted line,* dog that received no Lu-Tex. *Solid line,* dog that received 2 mg/kg Lu-Tex. Fluorescence measurement was taken before light delivery. *Dashed line,* dog that was given 2 mg/kg Lu-Tex. Fluorescence measurement taken after light delivery. The fluorescence spectra taken before light delivery (*solid line*) is qualitatively similar to the spectra obtained in the intralipid phantom. A decrease in the fluorescence peak was observed after light delivery.

quired for patients treated with IP PDT (6, 15). Furthermore, damage to the bowel was a major dose-limiting toxicity in the Phase I trial of Photofrin-mediated IP PDT (6, 7). Bowel anastomoses were performed at the time of laparotomy in four dogs. All anastomoses healed normally without stricture formation or anastomotic leak. No enhanced toxicities were observed in dogs who underwent a bowel resection before IP PDT in this study.

Histological review of biopsies taken at the time of laparoscopy revealed no clear PDT-related abnormalities. Mild-to-moderate peritoneal fibrosis and mild hepatic changes were observed in the laparoscopic biopsies in both treated and control dogs. The histological changes in liver were mild. These were observed in control dogs who received light only, and there was no PDT dose-response relationship noted. Dogs 11, 12, and 13 were treated with the highest dose of Lu-Tex and light but did not demonstrate any liver biopsy abnormalities (Table 3); although two of those dogs (12 and 13) did have mild LFT abnormalities (Table 2). Tissue samples taken at the time of necropsy also failed to show clear PDT-related abnormalities. The mild and moderate kidney changes were found only in IP PDT-treated dogs and may have been related to PDT. However, these histological findings were not associated with any clinical toxicities.

Several factors regarding the study design are important to consider. First, a 3-h photosensitizer-light interval was chosen because this schedule produced the greatest tumor efficacy in a s.c. murine tumor model (16). It should be noted that tumor-to-normal tissue selectivity in this murine model is not maximal at the 3 h photosensitizer-light interval (16). Therefore, based upon previous studies, one would expect substantial amounts of the photosensitizer to be present in normal tissues, and that this schedule might result in greater normal tissue toxicities compared with longer photosensitizer-light intervals. It is encouraging that significant normal tissue toxicity was not observed with the treatment regimen used in this study.

A second point regarding this study design is that the highest 730-nm light dose used, 2 J/cm^2, is similar to the maximally tolerated dose of 514 nm light found in a Phase I trial of Photofrin-mediated PDT in humans (6). These light fluences cannot be directly compared for many reasons, including: (*a*) differences between canine and human normal tissue tolerance; (*b*) differences in the photochemical properties of Photofrin and Lu-Tex; (*c*) differences in the light dosimetry systems used; and (*d*) differences in the tissue penetration of the two wavelengths of light. However, it is reasonable to assume that the doses of photosensitizer and light used in this study are clinically relevant given the knowledge that exists regarding IP PDT and Lu-Tex. Although the canine model is not completely predictive of human toxicity, the demonstration of safety at clinically relevant doses of photosensitizer and light provides information regarding the initial dose range of light and photosensitizer that could be used in a human Phase I clinical study.

Lu-Tex-mediated IP PDT was administered in this canine study with an attempt to perform both light dosimetry and photosensitizer measurements. The efficacy and toxicity of PDT are dependent upon the amount of light and photosensitizer present in the target tissue. The biological effect of a dose of light and photosensitizer in tissues is affected by many parameters, including drug delivery, the geometry of the treatment volume, photobleaching, tissue oxygenation, and the heterogeneity of tissue optical properties. Unfortunately, the present indications for PDT in the United States have not included rigorous light and photosensitizer dosimetry. Efforts to measure photosensitizer and light within tissues should aid in the development of a paradigm for predicting a biological effect based upon the amount of photosensitizer and light present in the target tissue (28, 29).

A method for measuring photosensitizer concentration in tissues that does not involve a biopsy and labor-intensive laboratory methodology is highly desirable in the clinic. *In situ* fluorescence-reflective measurements in abdominal tissues were made to determine whether this approach was feasible for future clinical trials. The fluorescence spectra of the canine bowel taken *in situ* demonstrated that this technique is feasible in the clinic and may be a valuable tool for future clinical studies. It is not as yet clear that this technique will be as valuable as a quantitative measure of photosensitizer concentration. Future studies will focus on correlating the fluorescence spectra measurements with tissue levels of Lu-Tex, as measured by the traditional high-performance liquid chromatography method.

In summary, Lu-Tex-mediated IP PDT at clinically relevant doses was well tolerated in this canine model. No serious PDT-related toxicities were observed. These findings support the continued development of Lu-Tex as a photosensitizer for IP PDT.

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