Studies of a Novel Photosensitizer Palladium-Bacteriopheophorbide (Tookad®) for the Treatment of Prostate Cancer

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ABSTRACT

In this study, photodynamic therapy (PDT) mediated with a novel, second generation photosensitizer Tookad® (palladium-bacteriopheophorbide, WST09, STEBA Biotech, France), is investigated as an alternative modality in the treatment of prostate cancer. *In vivo* normal canine prostate and spontaneous advanced prostate cancer are used as the animal model. PDT was performed by irradiating the surgically exposed prostates with a diode laser (763 nm, 150 mW/cm) to activate the i.v. infused photosensitizer. The effects of drug concentration, drug-light interval, and light fluence rate on the PDT efficacy were studied. The prostates and adjacent tissues (bladder and underlying colon) were harvested and subjected to histopathological examination. During the one-week to 3-month period post PDT treatment, the dogs recovered well with little or no urethral complications. Prostatic urethra and prostate adjacent tissues (bladder and underlying colon) were well preserved. Light irradiation delivered during drug infusion or within 15 min post drug infusion induced the similar extend of damages. PDT induced prostate lesions in both normal and cancerous prostate were characterized by marked hemorrhagic necrosis and atrophy. Maximum lesion size of over 3 cm in dimension could be achieved with a single 1-cm interstitial treatment, suggesting the therapy is very effective in ablating cancerous prostatic tissue. In conclusion, the second generation photosensitizer Tookad® mediated PDT may provide an effective alternative to treat prostate cancer.

Keywords: Photodynamic therapy, prostate cancer, tissue response, Tookad-PDT, vascular effects

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1. INTRODUCTION

In the United States, prostate cancer is the most common male cancer and the second leading cause of cancer death among men (1). Surgery, radiation and hormone therapy or their combinations are common treatment options used to remove or destroy cancerous tissue. Significant side effects, such impotency, urinary incontinence and injuries to nearby structures, are reported to be associated with these therapies (2, 3). Other approaches, such as cryotherapy, hyperthermia and high intensity focused ultrasound (HIFU) or focused extracorporeal pyrotherapy, have been developed for the treatment of localized prostate cancer (4-7). However, available data indicate that the results from these modern modalities are comparable with both radiotherapy and radical prostatectomy (4, 8). An alternative or adjunctive therapy to treat localized prostate cancer is thus desired, either for a primary cancer or recurrent cancer.

Photodynamic therapy (PDT) is an increasingly practiced cancer treatment modality, involves administrating a photosensitizer drug then activating the drug with light of the appropriate wavelength. Activating the photosensitizer initiates a sequence of photochemical; chemical and biological reactions, which ultimately lead to cell and tissue damage (9). PDT can destroy tumor by inducing apoptosis, direct killing of tumor cells, destruction of tumor blood supply and tumor vasculature, and activation of inflammation and immune responses. PDT utilizing the first generation photosensitizer, Photofrin (Axcan, Montreal, Canada), has been approved by the US Food and Drug Administration (FDA) for treatment of some types of malignant diseases. The feasibility of using PDT to treat prostate cancer on animal models has been investigated in this laboratory as well as several others (10-13). Canine prostate has been used as an animal model for studying PDT in prostate due to its resemblance both in physical size and in anatomical structure to that of human. Canine prostate tissue-responses to PDT mediated by various photosensitizer agents were investigated (14-20). Previous studies indicate that PDT is an effective and feasible modality in total ablation of the prostate gland; however, the optical doses in these studies were more or less empirically based on other pre-clinical studies. None of the referenced *in vivo* canine studies have utilized *in situ* dosimetry or reported *in vivo* optical properties of the prostate tissue, leaving an open question of how much of the variations in PDT induced damage were results of inadequate optical dosimetry. Although PDT has the advantage of being a highly localized therapy, there is a lack of data to address the ultimate question: how a PDT treatment should be delivered to achieve a specific goal, e.g. total ablation of the gland while maximally preserving the adjacent normal structures. This requires accurate dosimetry, novel treatment planning techniques and *in situ* treatment control to produce a predictable volume of prostate ablation.

The present work is a preclinical study utilizing a second-generation photosensitizer, Tookad® (Palladium-Bacteriopheophorbide, also known as WST09) to ablate prostatic tissue. Our previous study (10, 11) showed that Tookad-PDT can destroy a clinically-significant volume of prostate tissue with preservation of adjacent tissues. The objective of this study is (*a*) to further evaluate the drug/light dose escalating effects; (*b*) to determine the optimal drug-light intervals; (*c*) to test the efficacy of Tookad-PDT on prostate cancer. Although, many parameters regarding the

optimal photosensitizer and light combination to give maximum ablation of malignant tissue still remain to be elucidated, the results obtained from this and the previous study have provided a valuable data for the design of ongoing Tookad-PDT human clinical trials.

2. MATERIALS AND METHODS

2.1 Animal model A total of 24 adult healthy Beagles (2 ~ 9 years old, 10 ~ 18 kg) were studied. The preliminary data of the first 16 animals has been reported in previous publication (10, 11). The animals were obtained from licensed vendors (Marshall Farm, North Rose, NY, USA or Harlan Farm, Indianapolis, IN, USA) and conditioned for one week before any experimental procedures were carried out. All studies were performed under the guidance of the Institutional Care and Use Animal Committee at HealthONE Alliance and Colorado State University. The prostate in these animals is typically ~ 3 cm in lateral diameter. Three animals revealed chronic prostatitis and one hyperplasia at necropsy.

2.2. Premedication In the previous study, Benadryl (i.v. 0.7 ~ 1.4 mg/kg) and Dexamethasone (SQ, 2 mg per dog) were given immediately prior to the photosensitizer infusion (n = 14) to counteract the effect of the co-solvent Cremophor EL-P on blood pressure (11). Alternatively, the same drugs were given 24 hours prior to the photosensitizer infusion in this study (n = 9). Their effect on blood pressure drop was compared.

2.3. Photosensitizer Photosensitizer Tookad® (Palladium-Bacteriopheophorbide, also know as WST09, molecular weight 715, STEBA Biotech, France) was prepared in a Cremophor EL-P based vehicle by the manufacturer. The concentration of Tookad® was determined spectroscopically at 763 nm using extinction coefficient of 10.86×10^4 (21). In previous study, the drug concentration of 5.0 mg/ml was used. In this study, drug concentration of 2.5 mg/ml was used. Tookad® was given to the animal at dosages of 0.25, 0.5, 1 and 2 mg/kg, respectively, via a slow i.v. infusion (0.5 or 1 ml/min) through right cephalic vein catheter under the dimmed ambient lighting.

2.4. Light source, light delivery and light detection The light source was a portable 763 nm diode laser (CeraLas, CeramOptec, Bonn, Germany) with a maximum output power of 4 W and a calibration port for determining delivered output power. The laser output was directly coupled into a series of beam splitters (PDT Systems Model 1220, Santa Barbara, CA, USA. Fiber Splitter-400 micron, Ocean Optics Inc., Dunedin, FL, USA) that allowed up to 4 fields to be treated simultaneously. The light fluence was <=150 mW/cm for interstitial irradiations delivered through a diffuser tip of cylindrical fiber (10 mm active length, 1.3 mm diameter; Model CD 603-10C; CeramOptec, Germany). The diffuser tip was inserted in the middle of anterior section and approximately 1 cm away from the urethra. One animal received a transurethral irradiation (50 J/cm) delivered through a diffuser tip of cylindrical fiber. Tissue temperature and tissue optical measurement were carried out in 16 animals and the results were reported in previous publication (11).

2.5 Surgical and PDT Treatment Procedures Standard sterilization procedures were strictly followed. All surgical instruments were autoclaved and invasive probes chemically sterilized. As an extra precaution, the dogs received antibiotics before and after surgery (IM, Ampicillin, 20 mg/kg) to prevent possible infection. Pain control consisted of pre-operative and post-operative injection of morphine with long term control provided by Fentanyl patches. All dogs were prepared for surgery following a standard canine laparotomy procedure (10).

Tookad® (0 - 2 mg/kg) was administered by slow infusion through an i.v. catheter at a rate of 0.5 ml/min (drug concentration - 5 mg/ml) as reported previously (10) or at 1 ml/min (drug concentration - 2.5 mg/ml) over a period of 10 minutes. Light was applied during the drug infusion or 5 - 15 min after the completion of the infusion. In the case of 'during infusion PDT', light irradiation was terminated at the point of the completion of drug infusion. For interstitial irradiation 50, 100 or 200 J/cm was delivered. Irradiation lasted approximately 6 – 22 min in each animal.

Immediately following PDT treatment, the rectus muscle, fascia and skin were closed with interrupted sutures. The endotracheal tube was removed upon recovery of the swallow reflex. The i.v. catheter was either removed immediately after surgery or left for drawing blood samples. Standard procedures were followed for animal care, including continued pain control. Urinalysis was performed and results were reported in previous publication (11). After surgery the dogs were kept in dimmed ambient lighting for 2 – 4 h. The PDT treated animals and control animals (light or drug only) were euthanized at predetermined time points (1 week, 1 and 3 months) after PDT, using barbiturate overdose.

2.6 Prostate perfusion scan A synchronous vascular injection planning was performed on one dog prior to PDT, 2-day and 7-day post PDT. Helical CT images were acquired from the sacral promontory to the ischial tuberosities at 5 mm collimation with 1 sec scan time (120 kV and 125 mA; Picker PQ 2000, Philips, Bothell, WA, USA) prior to contrast injection. Following a rapid intravenous administration of non-ionic iodinated contrast medium (600 mg/kg, Hypaque-370, 5 ml/s), high temporal resolution scans (5 mm thick) were obtained at a single level of the prostate every 1.8 sec for 3 min. Images were analyzed using a CT Perfusion software (GE Medical Systems, Milwaukee, WI, USA).

2.7 Treatment of prostate cancer With owner consent, one dog (Beagle-Hound mix, 13.6 kg, ~12 years old) with spontaneous advanced primary prostate cancer, deemed unsuitable for other treatment, entered this study. The prostate was treated with interstitial Tookad-PDT. The dog was prepared for surgery and PDT following the standard canine laparotomy procedure described above. The enlarged cancerous prostate ($9 \times 10 \times 8$ cm) contained heterogeneous neoplasm mass and cysts. The left lobe was larger than the right lobe. Three cylindrical fibers with a diffuser tip size of 2, 2.5 and 5 cm were placed in the left lobe and one with a diffuser tip size of 2.5 cm placed in the anterior area of the right lobe. The distance between each fiber was 3 cm. Tookad® (drug concentration - 5 mg/ml, drug dose - 2 mg/kg) was administered by slow infusion (0.5 ml/min) through an i.v. catheter. Light was applied 5 min after the completion of the infusion. Each treatment area received a total light fluence rate of 100 – 150 J/cm over the period of 20 – 40 min. Approximately 1/3 of enlarged prostate was treated. Two metastatic tumors, one inside mouth and one on the front leg,

were surgically removed at the time of PDT. The dog was survived one week post the surgery, with death due to disease and the prostate was harvested for histopathology examination.

2.8 Histopathologic examination At necropsy, the prostate, bladder and underlying colon section were removed and photographed. The prostate was dissected from the urinary bladder. All specimens were fixed in 10% neutral buffered formalin. The prostate, bladder, and colon were cut into 3 mm blocks, photographed, and embedded in paraffin. Sections of 5 μm thickness were stained with standard H&E and Dyer's Verhoeff Variation (22) to examine the histopathological changes. The preliminary data from the first 16 animals has been reported in previous publication (11).

3. RESULTS

3.1 Premedication and blood pressure control

It was known that Cremophor EL-P, a co-solvent of Tookad®, could cause a marked blood pressure drop in the dogs. This side effect was easily controlled by pre-medication with anti-histamine (Benadril) and steroids (Dexamethasone) prior to Tookad® infusion and the adjustment of anesthesia setting during PDT. In this study, 14 animals received Benadril and Dexamethasone immediately prior to Tookad® infusion and 9 out of 14 animals (64%) still showed blood pressure drop during PDT. A total of 9 animals received Benadril and Dexamethasone 24 hours prior to Tookad® infusion and only 2 out of 9 animals (22%) showed a mild blood pressure drop during PDT.

3.2 Post-surgical observations

Our previous study (16 animals) showed that all control animals that received either light only or drug only, and in the PDT treatment groups survived the 1-week to 3-month post-surgical period without incident. The surgical wound healed well in all treated dogs, with no post-surgical urethral complications. None of the dogs showed urinary retention and urinary catheterization was not needed. Urinalysis was performed for one control (drug only) and three treated dogs (received 50, 100 and 200 J/cm, respectively), all had trace blood in the urine samples within the first 24 – 48 h, but none required medical attention or treatment (11).

3.3 Histopathological findings.

3.3.1 Macroscopic findings

At one week post treatment, the PDT-induced lesions were characterized by acute hemorrhagic necrosis with patchy sub-capsular hyperemia and marked edema, as described in detail in previous publications (10, 11). The PDT-induced lesions were well delineated from the adjacent normal tissue, and the zone of necrosis increased with the increase of the delivered light fluence and drug dose (Fig. 1). The boundary of the lesions was sharply defined, suggesting a PDT response threshold. At the same drug dose and light fluence, three light irradiation modes (during infusion, 5 min or 15

min after the completion of infusion) produced a similar size of necrotic lesion. At three months post PDT, prostate retained its anatomical structure and shape but a large reduction in gland volume.

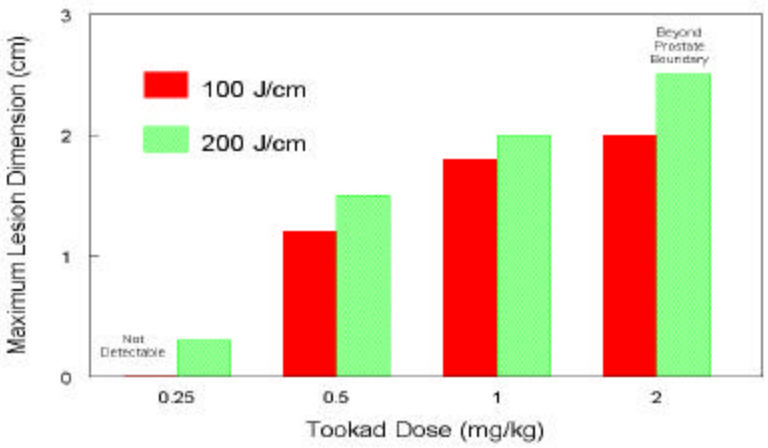

Figure 1. Lesion size as a function of photosensitizer dose. Note the chart is based on a single animal per data point.

3.3.2 Microscopic Findings

On H&E sections of both normal or prostatitic gland examined by light microscopy, marked hemorrhagic necrosis and atrophy of the glandular tissue in the treated areas, with local hemorrhagic vasculitis, were seen at one week post PDT. Tookad-PDT induced necrotic lesions could be detected in the lobes receiving high light fluence (200 J/cm) but very low drug dose (0.25 mg/kg). The fibromuscular region and connective tissue appeared to be damaged in the same way as the glandular tissue, characterized by diffuse and marked degeneration. Dyer's Verhoeff Variation stain showed that inside the PDT treated area the layers of collagenous and smooth muscle tissue of the capsule were preserved. However, collagen fibrils in subsidiary ductal and acinar parts of the prostate were completely destroyed by PDT (11). For cases (100 and 200 J/cm interstitial irradiation) in which the PDT lesion extended to the urethra, only mild submucosa congestion and small areas of epithelial disruption were observed in only some specimens. Collagen stain revealed ongoing healing at one month post PDT. At three months, PDT treated lobes showed necrosis had resolved completely (10, 11). The urethral structures, and fibromuscular and connective tissue of the capsule, appeared normal at one and three months in all animals.

3.4. Prostate perfusion scan.

Conventional CT scan showed changes in the prostate shape and size but did not show the PDT induced tissue damage. The blood perfusion scan performed at 48 hours and one week post PDT (1 mg/kg Tookad, left lobe - 100 J/cm and right lobe – 200 J/cm) clearly revealed PDT induced necrosis (Fig. 2). The right lobe showed a cavitary lesion. Severe hyperplasia and cavitation was confirmed during postmortem histology examination. Margins of necrosis areas can be determined without further image analysis. A detail perfusion analysis and mapping was performed with perfusion software. Results of blood perfusion analysis showed dramatic changes in blood flow, blood volume and permeability 48

h post PDT (data not shown). However, simple image analysis, such as a peak enhancement measurement with threshold, may be adequate for evaluating the changes in blood flow and blood volume after Tookad-PDT.

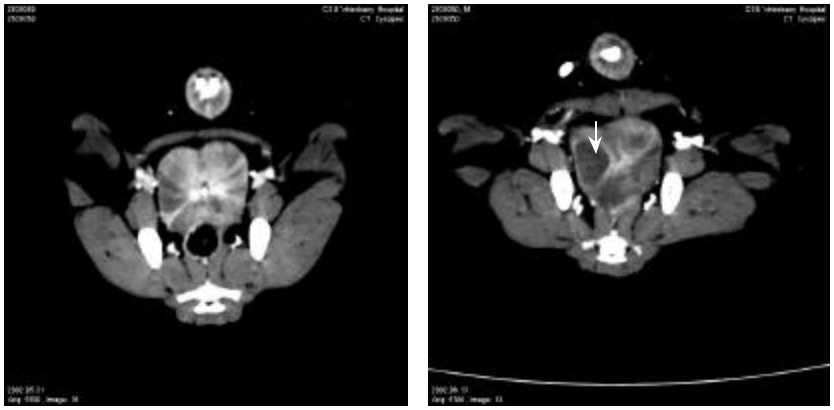

Figure 2. Blood perfusion scan. (Left): Pre-PDT scan and (Right): 48 hours post-PDT scan (right lobe – 200 J/cm and left lobe – 100 J/cm). The regions of PDT-induced necrosis are clearly visible and the lesion size correlates well with the light dose. Arrow indicates a cavitationary lesion.

3.5. Response of prostate cancer to Tookad-PDT

One dog suffering from spontaneous advanced primary prostate cancer was treated with Tookad-PDT. The dog survived one week post surgical and PDT procedures. Gross changes included visible necrosis and a 25% volume reduction in cancerous nodules of the treated area. Unfortunately, the prostate tumor had also spread outside the capsule and had invaded the soft tissues surrounding the prostate. Invasion of the urethra at the neck and into the bladder was responsible for the post-surgery urinary leakage. H&E stain showed the PDT induced severe necrosis of cancerous gland tissue, which indicated that a complete destruction of cancerous gland tissue could be achieved by Tookad-mediated PDT (Fig. 3). Although 1/3 of prostate underwent a palliative treatment with multiple diffuser fibers (n = 4) with long active tip (2 – 5 cm) in this particular case, the dog survived only one week due to disease progress.

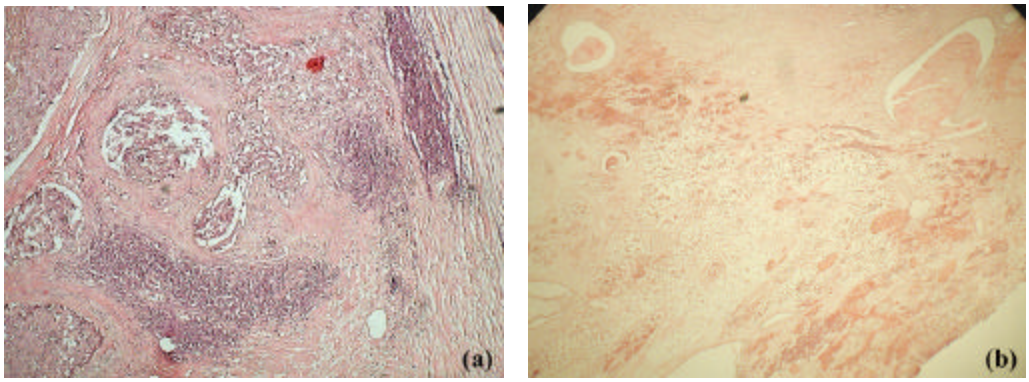

Figure 3. Response of prostate cancer to Tookad-PDT. (a): Cancerous area adjacent to PDT treatment zone, and (b): Necrotic zone of PDT-treated area. Tookad-PDT (2 mg/kg, 100 J/cm) induced a complete destruction of cancerous gland tissue. (Photographs are reduced from 40×).

4. DISCUSSION

Conventional prostate cancer therapies sometimes fail to provide satisfactory treatment results, while the side effects often significantly degrade the quality of life of patient. A localized cancer treatment modality, as an alternative in the management of prostate cancer and the maximal protection of surrounding structure, is therefore desired. The application of PDT to treat prostate cancer on animal models as well as human patients has been investigated for the past decade. However, the implementation of PDT in prostate cancer treatment is limited by the inaccurate or insufficient control of treatment volume, mainly due to poor optical dosimetry and slow pharmacokinetics of currently available photosensitizer(s). Our previous study (10, 11) suggests that Tookad-based PDT may overcome these problems and thereby provide an alternative/adjunctive modality to treat prostate cancer. This study further demonstrates the feasibility and effectiveness of Tookad-PDT for treatment of prostate cancer. The observations obtained from this study are directly relevant to designing and optimizing future clinical use of the Tookad-PDT for prostate cancer.

Canine prostate tissue-responses to PDT mediated by various photosensitizers have been investigated (14-20) and the general consensus is that, given a fixed optical dose, the value of dynamic light fluence and the volume of tissue damage are rather unpredictable. In contrast, the value of dynamic light fluence measured during Tookad-PDT is relatively stable. It is estimated that, with 200J/cm interstitial irradiation, 1.5 cm radius lesion, 3 mm attenuation depth and assuming a few mm range for fluence build-up due to backscatter, for the largest PDT lesions, an upper estimate of the light fluence at the PDT lesion boundary is ~ 20 $J.cm^{-2}$ (11). Nevertheless, it is also encouraging that the effect of Tookad-PDT on the urethra was minimal, both functionally and structurally, even when the urethra was within the treatment area and the periurethral prostate tissue was destroyed. This was not the case with Photofrin or other tested photosensitizers (16-20) and may be related to the Tookad® mechanisms of vascular targeting.

In contrast to many photosensitizers being investigated clinically for prostate cancer (23, 24), Tookad-PDT is believed to be purely vascularly mediated. The clearance of Tookad® from circulation is very fast and its plasma half-life is less than 1 h in a mouse model (A. Scherz, unpublished data). Similarly, our study also shows a rapid clearance of Tookad® in the canine model (data not shown). Consistent with this, rapid clearance has been observed in several other tissues (including tumor, skin) measured in *in vivo* models of other animals (25, B. Wilson, unpublished data). Bourre *et al* demonstrate in recent that there is little uptake of Tookad® by tumor but a gradual accumulation of Tookad® in liver in a mouse model (26). Wilson *et al* demonstrate that in both rat and pig models the skin phototoxicity disappears within a few hours of drug administration, even at high therapeutic doses (B. Wilson, manuscript in preparation). Hence, the post-treatment management should be considerably simplified for a patient receiving Tookad-PDT.

One of the advantages of Tookad-PDT is its being activated at relatively long wavelength (763 nm), with corresponding greater light attenuation depth (~ 4 mm) in prostatic tissue (11). Tookad-PDT induces much larger prostate lesions than

other photosensitizers. Figure 1 shows a drug/light dose escalating effect, that implies Tookad-PDT can be delivered under a precise control and therefore a predictable treatment outcome can be expected.

The nature of pure vascular effects of Tookad-PDT on prostate has been demonstrated, for the first time, by a simple perfusion CT scan. Tookad-PDT induces rapid and marked changes in blood flow and blood volume (Fig. 2). This demonstrates that Tookad-PDT can severely impair vascular architecture and shut down blood supply to the treatment region. Figure 3 shows the response of advanced prostate cancer to Tookad-PDT. Although the palliative treatment with multiple diffuser fibers was intended to relieve symptoms, the dog survived only one week due to disease progress. However, a complete destruction of cancerous tissue and reduction of prostate volume within the treated area was achieved with a total light dose of 100 – 150 J/cm. This demonstrates, for the first time, that Tookad-PDT can be effective to destroy prostate cancer, although a selective destruction of tumor is not prerequisite for PDT to be applied successfully in prostate cancer patients. Our early study reports mainly the response of normal prostate tissue to Tookad-PDT (10, 11). However, this study indicates that the normal and malignant prostatic tissue respond in similar manners. This is consistent with previous reports that if all other treatment parameters are identical, PDT is likely to be equally effective in destroying normal tissue and the embedded cancerous tissue arising from the same tissue origin (27-32). The advantage of PDT results in the rapidity of the healing process occuring in normal tissues.

It is known that Cremophor EL-P, the co-solvent of Tookad formulation for intravenous injection, can induce a marked blood pressure drop in the dogs, due to the anaphylactoid reaction in this species (33). This side effect can be easily controlled by pre-medication with anti-histamine (*i.v.* Benadril, 0.7-1.4 mg/kg) and steroids (Dexamethasone, 2 mg SQ) and adjustment of the anesthesia. However, earlier pre-medication, e.g. 24 h prior to Tookad-PDT, can significantly reduce the incidence of Cremophor EL induced blood pressure change. The administration of steroids might also play a role in preventing a potential urethral obstruction after PDT (11).

In conclusion, these results suggest that Tookad-PDT, utilizing a second-generation photosensitizer with an activation wavelength at the near-infrared, with a short waiting period between drug administration and light irradiation and fast photosensitizer clearance from the body, acting primarily upon vasculature, may provide an alternative/adjunctive modality to treat early stage primary prostate cancer and recurrent diseases.

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