SPIE, Proceedings Vol. 4612, pp. 29-39 (2002)

WST09 (TOOKAD) Mediated Photodynamic Therapy as an Alternative Modality in Treatment of Prostate Cancer

Qun Chen, Zheng Huang, David Luck, Jill Beckers, Pierre-Herve Brun, Brian C. Wilson, Avigdor Scherz, Yoram Salomon, Fred W. Hetzel (QC, ZH, DL, JB, FWH, HealthONE Alliance, Denver, CO 80218 USA) (AV, YS,Weizmann Institute, Rehovot, Israel) (PHB, NEGMA-LERADS, Toussus-Le-Noble, France) (BCW, Ontario Cancer Institute, Toronto, Canada)

ABSTRACT

Photodynamic therapy (PDT) utilizes optical energy to activate a pre-administered photosensitizer drug to achieve a localized tumor control. In the presented study, PDT mediated with a second-generation photosensitizer, WST09 (TOOKAD, Steba Biotech, The Netherlands), is investigated as an alternative therapy in the treatment of prostate cancer. *In vivo* canine prostate is used as the animal model. PDT was performed by irradiating the surgically exposed prostates both superficially and interstitially with a diode laser (763 nm) to activate the intra-operatively *i.v.* infused photosensitizer. During light irradiation, tissue optical properties, and temperature were monitored. During the one-week to 3-month period post PDT treatment, the dogs recovered well with little or no complications. The prostates were harvested and subjected to histopathological evaluations. Maximum lesion size of over 3 cm in dimension could be achieved with a single treatment, suggesting the therapy is extremely effective in destroying prostatic tissue. Although we found there was loss of epithelial lining in prostatic urethra, there was no evidence it had caused urinary tract side effects as reported in those studies utilizing transurethral irradiation.

In conclusion, we found second generation photosensitizer WST09 mediated PDT may provide an excellent alternative to treat prostate cancer.

Keywords: Photodynamic therapy, prostate cancer, optical dosimetry, tissue response, WST09(TOOKAD)

INTRODUCTION

In the United States, prostate cancer is the most common male cancer and the second leading cause of cancer death among men (1). While there are only palliative treatments for metastasized prostate cancer, early stage prostate cancer is often confined within the organ and is treated with curative intent with surgery and/or radiation therapy that offer a cure rate ranging from 50-70% (2-4). Significant side effects are reported to be associated with these currently available therapies. Approximately half of the patients suffer from impotency after either radiation or surgery (4,5). Other severe complications include urinary incontinence and injuries to nearby structures after radical prostatectomy (surgical removal of prostate organ) and infections of various nearby organs, and genital area swelling after radiation therapy. Furthermore, failure of radiation therapy appears to accelerate tumor growth and progression (4,5). Hyperthermia has been used in both benign hyperplasia and cancer of the prostate, primarily to relieve the urinary symptoms. The outcomes of the treatments appear to be modest (6,7). The primary limitation of hyperthermia is the difficulty to achieve a temperature (in the prostatic tissue) high enough to cause direct cell killing by the presently available hyperthermic devices. Other drawbacks of the modality are that a number of sessions are required to provide, even temporary, relief of the symptoms and a potential possibility of developing prostatorectal fistula (7). An alternative/adjunctive therapy to treat localized prostate cancer is thus desired.

Photodynamic therapy (PDT) is a new cancer treatment modality. A patient is administered a light-sensitive drug (photosensitizer). A waiting time is exercised to allow the drug to be absorbed/retained by target cells. The target is then irradiated with light of a proper wavelength. The light energy activates the drug and initiates a sequence of chemical and biological reactions, which ultimately lead to cell death (8). PDT utilizing the first generation photosensitizer, Photofrin, has been approved by the US Food and Drug administration (FDA) for treatment of selected types of tumors, such as esophageal and lung cancer.

The present work is a pilot study utilizing a second generation photosensitizer, WST09 (Palladium-Bacteriopheophorbide) to ablate prostatic tissue. With a total ablation of a prostate gland as the final goal, the current work concentrates on the biological response of a prostate and its adjacent normal tissue to a PDT treatment.

MATERIALS AND METHODS

Normal canine prostates were used as an *in vivo* animal model. A total of 16 Beagles were studied. The animals were obtained from licensed vendors (Marshall Farm, NY or Harlan farm, IN) and conditioned for a minimum of one week before any experimental procedures were carried out. All studies were performed under the guidance of the Institutional Care and Use Animal Committee (ICUAC).

Photosensitizer

Second generation photosensitizer WST09 (TOOKAD, Steba Biotech, The Netherlands (9, 10)) is a novel and pure Pd-substituted bacteriochlorophyll derivative (Fig. 1) and has a maximum absorption wavelength at around 760 nm (infrared) ($\varepsilon = 10^5$ $mole^{-1}cm^{-1}$in chloroform). The drug has an extremely fast pharmacokinetics and is usually cleared from the system within a few hours (10). The drug was given to the animal at a dosage of 2 mg/kg via a slow i.v. infusion.

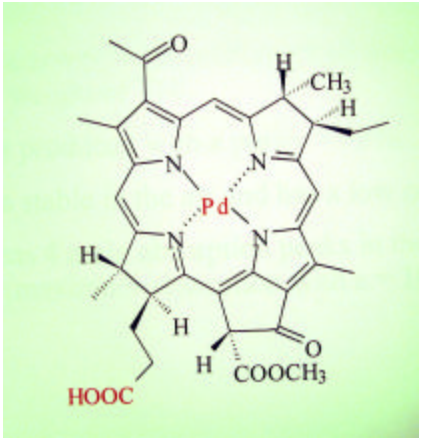

Figure 1. Molecular structure of WST09.

Light Source

Light source for PDT treatment and optical measurement was provided with a portable 763 nm diode laser (CeraLas, Ceramopteic, Germany) with a maximum output power up to 4 W. The laser output was directly coupled into a series of optical light splitters that allowed up to 4 irradiation fields to be treated simultaneously. The irradiation light fluence rates were controlled at equal or less than 150 mW/cm^2 for the superficial fields and 150 mW/cm for interstitial irradiations, respectively.

Surgical and Experimental Procedures:

Standard sterilization procedures were strictly followed for all in-vivo experiments. All surgical instruments and invasive probes were either autoclaved or sterilized in appropriate chemicals. As a precaution, the animals received antibiotics before and/or after surgery (SQ, Procaine Penicillin G, 20,000 units/kg) to prevent possible infection.

The animals are prepared for surgery following a standard canine laporatomy procedure. The incision for the surgery started midway between the costal margin and the umbilicus to the pubic symphysis. The connective and fat tissue around the prostate were dissected to expose the anterior and lateral prostate surface. Extra caution was taken during this step to preserve nerves. Once exposed, the prostate gland was raised for easier access by padding sterilized gauze under it. Optical fiber probes for PDT light irradiation and in site optical measurement were positioned into the prostate at pre-determined locations. For superficial irradiation, a 1 cm diameter light field was centered on a prostate lobe. For interstitial irradiation, a 1 cm length linear light diffuser was inserted into the opposite prostate lobe. The optical measurement probes were placed 3 or 6 mm radius from the interstitial fiber (Fig. 2). Thermocouples for temperature monitoring during PDT light irradiation were placed at 3 mm radius from the interstitial fiber or 1 mm below the irradiated surface, respectively. Control measurements were performed and biopsies sampled.

Photosensitizer WST09 (2 mg/kg) was infused into the animal through an *i.v* catheter at a rate of 0.5 ml/min. The total infusion time was between 10 and 14 min. Light was applied 5 min or 15 min after photosensitizer infusion (short drug-light interval was previously shown to be essential for having therapeutic effects with the new derivatives (10,11)). For superficial irradiation, light doses of 100 and 200 J/cm^2 were used. For

interstitial irradiation, a light dose of 150 J/cm was used. In addition to the direct irradiation of a prostate, adjacent normal structures of bladder and colon were irradiated simultaneously with the prostate, using separate optical fiber probes, to a controlled light dose up to 80 J/cm^2. Animals were kept in the dark during and after photosensitizer infusion. Light irradiation lasted between 8 - 23 min. After light treatment, the rectus muscle, fascia and skin were closed with interrupted sutures. The animals were injected with an analgesic such as morphine (0.5 mg/kg) or Bupremorphine (SQ, 0.3 mg/ml, 0.01-0.02 mg/kg) immediately after surgery and every 4 hours until the Fentanyl patch kicked in (approximately 12 hours after application). The endotracheal tube was removed upon swallow reflex. The I.V. catheter was removed and an Elizabethan collar was placed around the dog's neck to prevent licking of the incision site. Standard procedures were followed to care for the animals including continued pain control.

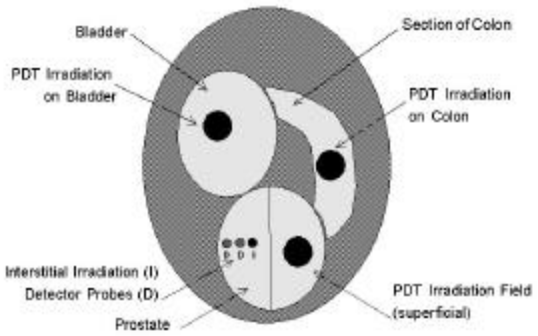

Figure 2: Experimental setup for PDT irradiation and monitoring. Note the probe positions for oxygen and temperature are not shown in the figure. They are placed either immediately under the superficially irradiated surface or approximately 3 mm radius from the interstitial irradiation source.

The PDT treated animals were terminated at predetermined time points (one week, one month and three months, respectively) after the light treatment, by using barbiturate overdose. The prostate, bladder and colon were removed for histopathological evaluation of the PDT induced lesion sizes.

Histopathology Examination:

At necropsy, the prostate, bladder, and colon were removed and photographed. All specimens were fixed in 10% neutral buffered formalin. The prostate was dissected from the urinary bladder. The prostate, bladder, and colon were cut into 3 mm blocks, photographed, and embedded in paraffin. Sections of 5 μm were stained with H&E to examine the histopathological changes.

RESULTS

Thermal Effect

With 150 mW/cm^2 superficial or 150 mW/cm interstitial irradiation fluence rate, we observed no thermal effect (0.2 – 0.9 ^{o}C increasing during light irradiation) at either 3 mm radius from the interstitial irradiation

source or 1 mm below the superficially irradiated surface. It is thus concluded that all the damage, as observed in these animals, was induced by PDT. It was further confirmed that there was no damage in the control prostate that received only light irradiation (200 J/cm^2 or 200 J/cm) but no drug.

Dynamic Light Fluence Distribution during PDT

Figure 3 is representative of light fluence at a fixed location near the interstitial irradiation source during a PDT treatment. At each of the measurement sites (3 or 6 mm radius from the source, respectively), the light fluence remained relatively constant throughout PDT light irradiation. Consequently, the calculated effective attenuation depth as a function of time remained stable (Fig. 3). Small variations in light fluence rate, observed in the studies, are likely due to minor movement of the relative positions of the probes and the gland rather than true optical phenomena. The stable optical properties of a prostate during WST09 mediated PDT suggests that optical dosimetry is less unpredictable at the infrared region than that in 630 nm range.

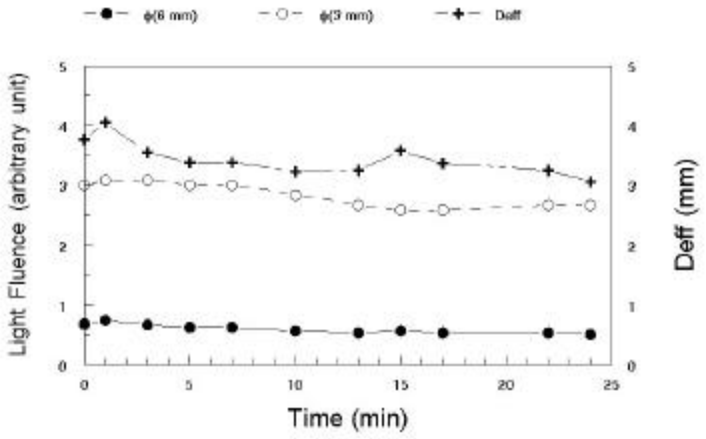

Figure 3. Dynamic light fluence rate and attenuation depth (Deff) during a 763 nm WST09 PDT treatment.

Post Surgical Observation of the Animals

All animals that received either light or drug only (control) or PDT treatment survived from 1-week to 3-month post-surgical period without incident. During the post-treatment period, none of the dogs showed urinary retention. No urinary catheterization was needed in any of the animals. Out of the 13 treated-animals, 3 had trace blood in urine within the first 24 hours after the treatment that later healed without further incident. None required medical attention or treatment. The surgical wound healed nicely with no signs of any post-surgical complications.

Macroscopic Findings upon Euthanasia

In a total of 6 dogs, each lobe received single interstitial irradiation (one diffuser fiber placed in the left lobe and one placed in right lobe of each prostate). In another total of 7 dogs, each lobe received one superficial irradiation or one interstitial irradiation, respectively. The single fiber irradiation delivered partial gland

treatment that allowed an accurate evaluation of lesion size. In the control group, three dogs each received 2 mg/kg drug or 200 J/cm or 200 J/ cm^2 light only. The control group showed no visible abnormalities.

Interstitial irradiation

A group of 4 dogs (total of 5 lobes) received 2 mg/kg and 50 J/cm light dose. Three dogs were terminated at one week after treatment and one dog at one month. At one week, the lesion was characterized by acute necrosis with patchy sub-capsular hyperemia and edema. The lesions were centered in the lobe. The capsular structure of the prostates appeared intact. The maximum lesion diameter was greater than 15 mm, with a depth of 15 mm. There was no significant difference in lesion diameter between 5 min and 15 min drug-light application intervals. The urethral structure appeared normal. At one month, the gland appeared pale and the lesions appeared to be resolving significantly (Fig. 4). Residual lesion size was small and still noticeable but surrounded with resolving necrosis throughout the entire (small) gland.

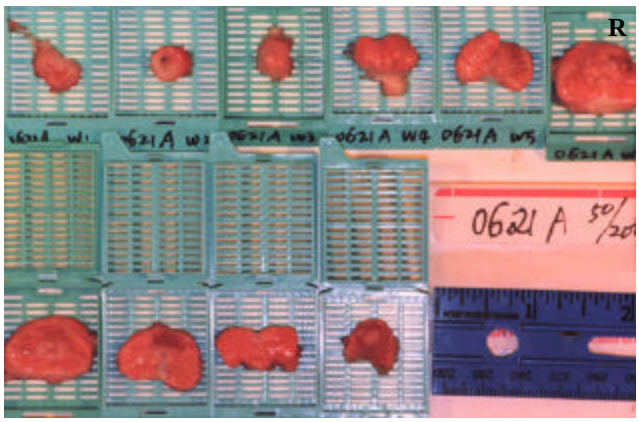

Figure 4. Dissection view of PDT lesion at one month. Prostate received 50 J/cm (left lobe) and 200 J/cm (right lobe) interstitial irradiation, respectively. Residual necrosis, periurethral damage and gland volume reduction were noticed.

A group of 6 dogs (total of 6 lobes) received 2 mg/kg drug and 100 J/cm light dose. Five dogs were terminated at one week after treatment and one dog at three months. At one week, the lesion was characterized by acute necrosis with patchy sub-capsular hyperemia and marked edema. The maximum lesion diameter was about 20 mm with the depth of 15 - 27 mm. There was no significant difference in lesion diameter between 5 min and 15 min drug-light application intervals. At three months, the gland appeared normal, but pale. Volume reduction was obvious. The lesions appeared to be resolving significantly (see Fig. 4 & 5). Residual lesion size was small but noticeable and surrounded with resolving necrosis throughout entire gland.

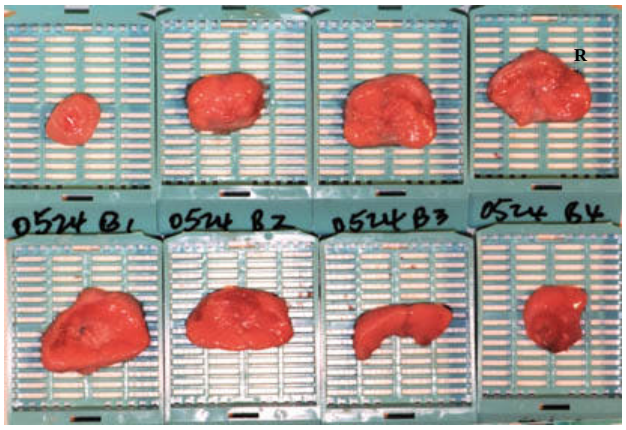

Figure 5. Dissection view of PDT lesion at three months. Prostate received 100 J/cm (left lobe) and 200 J/cm (right lobe) interstitial irradiation, respectively. Residual necrosis, periurethral fibrosis and gland volume reduction were visible at right lobe.

A group of 8 dogs (total of 8 lobes) received 2 mg/kg drug and 200 J/cm light dose. Six dogs were terminated at one week after treatment and one dog at one month and one at three months. At one week, the lesion was characterized by acute necrosis with patchy sub-capsular hyperemia and marked edema. The maximum lesion diameter was greater than 23 mm with the depth of 24 - 39 mm. There was no significant difference in lesion diameter between 5 min and 15 min drug-light application intervals. Some dissected sections showed that lesions covered the entire cross-section around the treatment site, and complete destruction of prostatic and urethral tissue. At one month, the gland appeared pale and smaller. The lesions appeared to be resolving (see Fig. 5). Residual lesion size was noticeable and surrounded with resolving necrosis throughout the entire gland. The urethral lesion was visible. At three months, the gland appeared normal, but pale and smaller. Volume reduction was obvious. The lesions appeared to be resolving significantly (see Fig. 4). Residual lesion size was small but noticeable and surrounded with resolving necrosis throughout the entire gland. The urethral lesions were still visible.

Superficial irradiation

Prostate: A group of 3 dogs (total of 3 lobes) received 2 mg/kg drug and 100 J/cm^2 light dose. At one week after treatment, the lesion was characterized by acute necrosis with patchy sub-capsular hyperemia and marked edema. The lesion was located at the upper quadrants of each irradiated lobe. The lesion appeared as well-circumscribed necrosis. The maximum lesion diameter was 14 mm with the maximum depth of 24 mm. Some dissected sections showed that lesions covered the entire cross-section around the treatment site, and complete destruction of prostatic tissue. The urethral structures were intact. A group of 4 dogs (total of 4 lobes) received 2 mg/kg drug and 200 J/cm^2 light dose. At one week, the lesion was characterized by acute necrosis with patchy sub-capsular hyperemia and marked edema. The maximum lesion diameter was greater than 21 mm with the depth of 30 mm. The lesions extended from the surface deep into the tissue (2-3 cm). The lesions were mainly located at the upper quadrants of each irradiated lobe. Some dissected sections showed that lesions covered the entire cross-section around the treatment site, and complete destruction of prostatic tissue. The urethral structure appeared unaffected.

Bladder and Colon

There was no visually observable damage on the bladder or colon when treated with a light dose up to 40 J/cm^2. The section of the colons that received 80 J/cm^2 light irradiation showed marked superficial hemorrhage. Ulceration corresponding to the irradiation field was clearly visible. There was no visually detectable perforation. Given a centrally delivered irradiation dose of 200 J/cm on prostate, it is unlikely that the periphery of the prostate would receive more than 40 J/cm of irradiation based on the effective attenuation coefficient obtained from the animal. Since such a light dose was powerful enough to destroy the entire cross section of a prostate, it is unlikely more energy would be necessary for a practical prostate ablation. We thus conclude that the therapy is rather safe for the adjacent normal structures.

With a single interstitial and a superficial irradiation for each lobe, at the given level of light and drug dose, PDT treatments did not eradicate an entire prostatic gland of older dogs (e.g. ~ 8 years old). In these dogs, gland tissue in the lower quadrant of each prostate was unaffected by PDT. The result was within our expectation that a multi-fiber implantation is likely to be the approach for total prostate ablation. Such an arrangement would also benefit the preservation of adjacent structures since a prostate is not a perfectly round object but rather has a smooth oval shape.

It is worth noting that within the sections near the irradiation sources, the lesions covered the entire treated lobes thus also the prostatic urethra. This is very important since transurethral irradiation, as reported by others (12), was a major reason for post-surgical complication such as urinary retention and serious hematuria. If a total prostate ablation can be achieved with multiple implantation of interstitial irradiation sources with the urethra situates at the margins of the PDT induced damage, it is likely such urinary tract complications can be significantly reduced or avoided.

Light Microscopic Examination

Prostate

No abnormalities were noticed in either the light only control dogs that were terminated at 1 week or the drug only control dog that was terminated at 3 months. Microscopic examination confirmed that extended lesions were induced by the PDT treatments. In the PDT treated dogs, at one week there was marked hemorrhagic necrosis of the glandular tissue in the treated areas with local hemorrhagic vasculitis. The size of the lesions correlated well with the light doses. The lesion boundary also corresponded well with the visually observed hemorrhagic lesions. At the peripheral zone of the prostate, distal from the irradiation sources, normal glandular structures were found to be well preserved, and some were surrounded with atrophic prostatic acinar tissue. There was a clear demarcation between the lesions and the preserved normal structures. The fibromuscular connective tissue appeared to be damaged in the same way as the glandular tissue, characterized by diffuse and marked necrosis. The prostatic capsule was mainly unaffected. At 100 J/cm and 200 J/cm interstitial irradiation, periurethral glandular and connective tissue appeared to be affected, in some cases the lesion spread to urethral epithelium layer. A small dimension of submucosa congestion and epithelium disruption was observed in some specimens. This might be due to the light overlap on the region from two interstitial irradiations or the probe was placed too close to the urethra and periuretha. At 50 J/cm interstitial irradiation and 200 J/cm^2 superficial irradiation, periurethral glandular and connective tissue appeared to be unaffected. At one month, the areas treated with 50 J/cm and 200 J/cm interstitial irradiation showed resolving necrosis with atrophic glandular tissue interposed with hemorrhagic necrosis. There was little glandular regeneration. At 3 months, the areas treated with 100 J/cm and 200 J/cm interstitial irradiation

showed resolving necrosis with persistent glandular atrophy interposed with residual hemorrhagic necrosis. Areas of prostatic capsule showed inflammatory reaction. There was little glandular regeneration.

Bladder and Colon

Under light microscopy, minor hemorrhage was observed at the surface of bladders irradiated with 40 J/cm^2. There were no clear indications of necrosis. No abnormalities were observed on the bladder and bladder neck while the prostate gland received up to 200 J/ cm^2 superficial irradiation or 200 J/cm interstitial irradiation, therefore, the risk of fistula formation was very low at a high light dose level.

Similarly, we observed only slight inflammatory reactions on the colon surface that was irradiated with up to 40 J/cm^2 PDT. The mucosa and underlying colonic glandular structures were well preserved with 40 J/cm^2 irradiation. The section of colon that was irradiated with 80 J/cm^2 PDT light dose showed marked hemorrhagic damage at the surface one week after the treatment. Full depth necrosis was observed within the irradiation field of these specimens. No adverse effect was observed on colonic or rectal mucosa when the prostate received up to 200 J/cm^2 superficial irradiation or 200 J/cm interstitial irradiation. In the three animals where the colon was stitched directly to the prostate to simulate a human anatomic structure, we observed no histopathological changes on the prostate-contacting region. Therefore, the risk of fistula formation was very low.

DISCUSSION

For early stage prostate cancer that is confined within the prostate organ and is often multifocal, conventional therapies such as ionizing radiation or surgical prostatectomy do not differentiate between normal and cancerous prostate tissue. Rather, the goal is to ablate/remove the entire organ. Thus, the goal of PDT in the management of prostate cancer, is likely to be total ablation of the gland (13). There are reports in literature indicating that, if all other treatment parameters are identical, PDT is likely to be equally effective in destroying normal tissue and the embedded cancerous tissue arisen from the same tissue origin (14-19).

Since prostate cancer is a very slow growing disease, it is possible that PDT may be used as a primary modality to treat prostate cancer. The advantages of using PDT in place of radiation or surgery are: reduced side effects, better localization and a single minimally invasive intervention. It has been well defined that PDT, in general, destroys glandular tissue, either normal or neoplastic, yet has little effect on connective tissue (20,21). That is confirmed by our current investigation, that a prostate can largely retain its anatomical shape and structures, even through a WST09 mediated PDT treatment destroyed a significant portion of its glandular structure. Furthermore, the healing process of a prostate treated with WST09 mediated PDT is largely by tissue regeneration that makes it less prone to side effects, compared to those more direct interventions such as surgical prostatectomy.

The feasibility of using PDT to treat prostate cancer on animal models has been investigated in this laboratory as well as several others. The effectiveness of PDT on cell destruction depends on multiple factors, including intrinsic cell sensitivity, local tissue concentration of the photosensitizer, absorbed light dose and the availability of molecular oxygen. There are several reports of PDT treatment in a rat prostate tumor model (22-24). The conclusion from these rat models is that PDT can effectively kill prostate tumors. Canine provides an excellent animal model for studying PDT in prostate due to its close resemblance both in physical size and in anatomical structure to that of human. *In-vivo* optical properties of canine prostate have been studied for Photofrin mediated PDT in this laboratory and found to be similar to that of *in-vivo* human prostate (13,25,26). Canine prostate tissue-responses to PDT mediated by various photosensitizer agents

(Photofrin and SnET2) were investigated and the general consensus is that, given a fixed optical dose, the volume of tissue damage is rather unpredictable. In comparison to the first generation photosensitizer Photofrin, most second generation photosensitizers have faster tissue-clearance time. Nevertheless, a PDT session with these drugs still has to be divided into drug infusion, waiting, and light irradiation phases, which require up to several days to complete (27-31).

The photosensitizer, WST09, used for the current study, is a unique drug. Unlike PDT mediated by Photofrin or most other second-generation photosensitizers, WST09 mediated PDT achieves its effect mainly *via*. vascular damages (10). The localization of the therapy is possible in a timely delivery of the photosensitizer and a precise optical dosimetry. Intracellular uptake of the photosensitizer, if any, has a minimal effect on the outcome of the WST09 mediated PDT. A distinctive characteristic of WST09 from other second generation photosensitizers is its rapid pharmacokinetics. The drug is *i.v.* infused into a patient and reaches the target through vascular circulation. The activation of the drug is accomplished while the drug is in the vascular circulation. Unlike PDT mediated with other photosensitizers which all heavily rely on a timely cellular uptake of the drugs, there is practically no waiting period necessary between the drug infusion and the start of light irradiation in a WST09 mediated PDT session. A WST09 mediated PDT treatment can thus be combined into one single session with far reduced inconvenience to the patients. The fast clearance of the drug from a patient body further improves the potential usefulness of the therapy by significantly reducing the time required for a patient to stay out of direct sunlight over a long period of time, thus causes less interruption to a patient's life style after a treatment.

There are no peer-reviewed publications in the literature utilizing PDT to treat human prostate cancer. The preliminary animal studies conducted in this laboratory using WST09 as a photosensitizer to ablate prostate tissue is deemed very effective and predictable. In comparison to that observed at 630 nm, the 763 nm light transmission in prostate was far less prone to the mechanical damage or changes in blood perfusion during an interstitial PDT irradiation (9). WST09, infused intra-operatively, clearly induced certain side effects (i.e. a marked drop in animal blood pressure). This side effect was easily controlled by a pre-medication of anti-histamine (i.v. Benadril, 0.7-1.4 mg/kg and Steroid Dexamethasone, 2 mg SQ) and adjustment of anesthesia settings. No irreversible damage was observed due to the photosensitizer itself.

Our study demonstrates that the size of WST09-mediated prostate lesion correlates well with the light dose delivered by a single irradiation fiber. The threshold light dose for superficial irradiation and 2 mg/kg drug dose might be between 20 and 40 J/cm^2. It is impressive that all interstitial light dose ranges between 50 – 200 J/cm produced severe tissue necrosis. There was little glandular regeneration at one month or three months. Gland volume reduction was visible at 3 months (though no quantitative measurement was performed). The new drug allows PDT treatment to start shortly (e.g. 5 min) after drug infusion. Therefore, the time required for WST09-mediated PDT irradiation can typically be completed within a few hours – a significant reduction of overall treatment length. In comparison to the other photosensitizer investigated in this laboratory and reported by others, WST09 is likely to become an ideal photosensitizer for the purpose of total prostate ablation in the treatment of prostate cancer, due to its deep light penetration, fast acting pharmacokinetics, and extensive tissue damage. No adverse effect was seen on the surrounding tissues, such as bladder and colon/rectum, when prostate received up to 200 J/cm^2 superficial irradiation or 200 J/cm interstitial irradiation. With a thorough investigation of the tissue optical properties, WST09 will likely provide an ideal modality for the treatment of prostate cancer via total prostate ablation.

REFERENCES

1. Boring, C.C., Squires, T.X., Tong, T., et al (1994) Cancer Statistics 1994. *Ca. J. Clin*. **44,** 7.
2. Paulson, D.F. (1988) Randomized series of treatment with surgery versus radiation for prostate adenocarcinoma. In Consensus Development Conference on the Management of Clinically Localized Prostate Cancer. NCI Monographs. No. 7. (Edited by Wittes, R.I.), pp. 117-122. Government Printing Office, Washington DC, (NIH publication No. 88-3005).
3. Lepor, H. and P.C. Walsh (1988) Long-term results of radical prostatectomy in clinically localized prostate cancer: experience at the John Hopkins Hospital. In Consensus Development Conference on the Management of Clinically Localized Prostate Cancer. NCI Monographs. No. 7. (Edited by Wittes, R.I.), pp. 117-122. Government Printing Office, Washington DC, (NIH publication No. 88-3005).
4. Catalona, W.J. (1994) Management of cancer of The Prostate. NEJM **331,** 996.
5. Stamey, T.A. (1993) Irradiation as primary treatment for prostate cancer. AUA Today **6**, 14.
6. Henderson, B.W. and T. J. Dougherty (1992) How photodynamic therapy works? Photochem. Photobiol. **55,** 145-157.
7. Chen, Q. and F.W. Hetzel (1998) Laser dosimetry Studies in the Prostate. J. Clin Laser Med Surg. **16,** 9-12.
8. Lee, L.K., Whitehurst, C., Pantelides, M.L. and J.V. Moore (1995) *In situ* comparison of 665 and 632 nm wavelength light penetration in the human prostate gland. Photochem. Photobiol., **62,** 882-886.
9. Scherz A., Salomon Y. Scheer H. and A. Brandis (1999) International PCT Patent Application No. PCT/IL99/00673, "Palladium-substituted bacteriochlorophyll derivatives and use thereof"
10. Brandis, A., Mazor, O., Gross, S., Koudinova, N., Hammi, R., Kälin-Kammhuber, N., Rosenbach-Belkin, V., Greenwald, M., Bondon, A, Simonneaux, G., Scheer, H., Salomon, Y. and Scherz A. (2001) Palladium-Bacteriochlorophyll derivatives: Synthesis and Phototoxicity (submitted to)
11. Zilberstein, J., Schreiber, S., Bloemers, M.C.W.M., Bendel, P., Neeman, M., Schechtman, E., Kohen, F., Scherz A. and Y. Salomon (2001) Anti vascular treatment of solid melanoma tumors with bacteriochlorophyll-serine-based photodynamic therapy, Photochem. Photobiol. **73**, 257-263.
12. Selman, S.H. and R.W. Keck (1994) The effect of transurethral light on the canine prostate after sensitization with the photosensitizer tin (II) etiopurpurin dichloride: a pilot study. J. Urol. **152,** 2129-2132.
13. Chen, Q., Wilson, B.C., Shetty, S.D., Patterson, M.S., Cerny, J.C. and F. W. Hetzel (1997) Changes in *In Vivo* Optical Properties and Light Distributions in Normal Canine Prostate During Photodynamic Therapy. Radiat. Res. **147,** 86-91.
14. Wagnieres, G., Hadjur, C., Grosjean, P., Braichotte, D., Savary, J.F., Monnier, P. and H. van den Bergh (1998) Clinical evaluation of the cutaneous phototoxicity of 5,10,15,20- tetra(m-hydroxyphenyl)chlorin. Photochem Photobiol **68,** 382-387.
15. van Geel, I.P., Oppelaar, H., Oussoren, Y.G., van der Valk, M.A. and F.A. Stewart (1995) Photosensitizing efficacy of MTHPC-PDT compared to photofrin-PDT in the RIF1 mouse tumour and normal skin. Int J Cancer. **60,** 388-394.
16. Chen, J.Y., Mak, N.K., Wen, J.M., Leung, W.N., Chen, S.C., Fung, M.C. and N. H. Cheung (1998) A comparison of the photodynamic effects of temoporfin (mTHPC) and MC540 on leukemia cells: efficacy and apoptosis. Photochem Photobiol. **68,** 545-554.
17. Regula, J., Ravi, B., Bedwell, J., MacRobert, A.J. and S.G. Bown (1994) Photodynamic therapy using 5-aminolaevulinic acid for experimental pancreatic cancer-prolonged animal survival. Br J Cancer **70,** 248-254.
18. Bedwell, J., MacRobert, A.J., Phillips, D. and S.G. Bown (1992) Fluorescence distribution and

photodynamic effect of ALA-induced PpIX in the DMH rat colonic tumor model. Br J Cancer. **65,** 818-824.

19. Barr, H., Krasner, N., Boulos, P.B., Chatlani. P. and S.G. Bown (1990) Photodynamic therapy for colorectal cancer: a quantitative pilot study. Br. J Surg. **77,** 93-96.
20. Koudinova, N., Pinthus, J. H. , Brandis, A., Mazor, O. , Brenner, O.,Bendel, P., Ramon J., Eshhar, Z., Scherz, A and Y. Salomon. (2001) Curative and Palliative Effects of a Novel Photodynamic Agent (Pd Bacteriopheophorbide) On Prostate Cancer: *In Vivo* Studies Using Human Prostate Cancer Xenografts. 8th Annual Scientific Retreat CapCURE Annual Retreat, Lake Tahoe Nevada, Sept. 2001.
21. Koudinova, N., Pinthus, J.H., Brandis, A., Brenner, O., Bendel, P., Ramon J., Eshhar, Z., Scherz, A and Y. Salomon (2001) TOOKAD Based Photodynamic Therapy: Successful *In Vivo* Treatment of Human Prostatic Small Cell Carcinoma Xenografts (submitted to).
22. McPhee, M.S., Thorndyke, C.W., Thomas, G., Tulip, J., Chapman, D. and W. H. Lakey (1984) Interstitial applications of laser irradiation in hematoporphyrin derivative-photosensitized Dunning R3327 prostate cancers. Lasers Surg. Med. **4,** 93-98.
23. Moore, R.B., Chapman, J.D., Mercer, J.R., Mannan, R.H., Wiebe, L.I., McEwan, A.J. and M.S. McPhee (1993) Measurement of PDT-induced hypoxia in Dunning prostate tumors by iodine-123-iodoazomycin arabinoside. J. Nucl. Med. **34**, 405-411.
24. Momma, T., Hamblin, M.R., Wu, H.C. and T. Hasan (1998) Photodynamic therapy of orthotopic prostate cancer with benzoporphyrin derivative: local control and distant metastasis. Cancer Res. **58,** 5425-5431.
25. Lee, L.K., Whitehurst, C., Pantelides, M.L., and J.V. Moore (1995) *In situ* comparison of 665 nm and 633 nm wavelength light penetration in the human prostate gland. Photochem. Photobiol. **62,** 882-886.
26. Lee, L.K., Whitehurst, C., Pantelides, M.L., and J.V. Moore (1995) Interstitial Photodynamic Therapy in the Canine Prostate. Br. J. Urol**. 80,** 898-902.
27. Selman, S.H., Keck, R.W. and J. A. Hampton (1996) Transperineal photodynamic ablation of the canine prostate. J. Urol. **156,** 258-260.
28. Selman, S.H., Albrecht, D., Keck, R.W., Brennan, P. and S. Kondo (2001) Studies of Tin Ethyl Etiopurpurin Photodynamic Therapy of the Canine Prostate. J. Urol. **165,** 1795-1801.
29. Chang, S., Buonaccorsi, G.A., MacRobert, A.J. and S. G. Bown (1997) Interstitial Photodynamic Therapy in the Canine Prostate With Disulfonated Aluminum Phthalocyanine and 5-Aminolevulinic Acid-Induced Protoporphyrin IX. The Prostate, **32,** 89-98.
30. Chang, S., Buonaccorsi, G., MacRobert, A. and S. G. Bown (1996). Interstitial and Transurethral Photodynamic Therapy of the Canine Prostate Using Meso-Tetra-(m-Hydroxyphenyl) Chlorin. Int. J. Cancer. **67,** 555-562.
31. Hsi, R. A., Kapatkin, A., Strandberg, J., Zhu, T., Vulcan, T., Solonenko, M., Rodriguez, C., Chang, J., Saunders, M., Mason, N. and S. Hahn (2001) Photodynamic Therapy in the Canine Prostate Using Motexafin Lutetium. Clinical Cancer Research, **7,** 651-660.