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Motexafin lutetium-photodynamic therapy of prostate cancer: Short and long term effects on PSA

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Abstract

Purpose: The time course of serum PSA response to photodynamic therapy (PDT) of prostate cancer was measured.

Experimental Design: Seventeen patients were treated in a Phase I trial of motexafin lutetium-PDT. PDT dose was calculated in each patient as the product of the *ex vivo*-measured pre-PDT photosensitizer level and the *in situ*-measured light dose. Serum PSA level was measured within two months prior to PDT (baseline), and at day 1; weeks 1-3; months 1, 2 and 3; months 4-6 and months 7-11 after PDT.

Results: At 24h after PDT, serum PSA increased by $98 \pm 36\%$ (mean $\pm$ SE) relative to baseline levels ($p=0.007$). When patients were dichotomized based on median PDT dose, those who received high PDT dose demonstrated a $119 \pm 52\%$ increase in PSA compared to a $54 \pm 27\%$ increase in patients treated at low PDT dose. Patients treated with high vs. low PDT dose demonstrated a median biochemical delay of 82 vs. 43 days ($p=0.024$), with biochemical delay defined as the length of time between PDT and a nonreversible increase in PSA to a value $\geq$ baseline.

Conclusions: Results show PDT to induce large, transient increases in serum PSA levels. Patients who experienced high PDT dose demonstrated greater short-term increase in PSA and a significantly more durable PSA response (biochemical delay). These data strongly promote the need for individualized delivery of PDT dose and assessment of treatment effect in PDT of prostate cancer. Information gained from such patient-specific measurements could facilitate the introduction of multiple PDT sessions in patients who would benefit.

Keywords

motexafin lutetium; prostate; PSA; PDT dose; photosensitizer concentration

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STATEMENT OF CLINICAL RELEVANCE

Treatment options for locally recurrent prostate cancer after radiation therapy are limited. Photodynamic therapy (PDT) is an attractive experimental modality because it offers the possibility of repeat treatments and minimal morbidity. Yet, there is considerable intra- and inter- patient variability in all aspects of PDT dosimetry including drug concentration, fluence rate, and optical properties. Therefore, the ability to perform individualized, *in situ* measurement of PDT dose is valuable, but the rapid assessment of biological response could significantly improve patient care by providing a means for the early identification of those who would benefit from additional treatment sessions. In the treatment of prostate cancer, PSA response may provide a simple, inexpensive monitoring tool with the potential to rapidly report on treatment effect, thereby facilitating the identification of patients who should be further considered, e.g through prostate biopsy, for subsequent PDT treatment sessions.

INTRODUCTION

Prostate cancer (PC) is the most frequently diagnosed cancer in men in the United States and the second leading cause of cancer deaths in men (1). PC is a multi-focal disease and control requires treatment of the entire gland (2). Since the introduction of Prostate Specific Antigen (PSA) monitoring as a screening tool, more patients are diagnosed at early stage of disease. Standard therapy options for local control of prostate cancer include radiation therapy (RT) by brachytherapy or external beam radiation, prostatectomy, and androgen deprivation. Unfortunately, treatment of patients with local recurrence after radiation therapy is challenging (3). Surgery after RT is limited by significant morbidity with high incidence of urinary incontinence, rectal injury, and impotence due to post radiation fibrosis (4,5). Androgen deprivation therapy is not curative and is poorly tolerated (3). New treatment approaches are needed for patients with locally recurrent prostatic disease.

Photodynamic therapy (PDT) involves the administration of photosensitizer followed by local activation of this drug with specific wavelengths of light. The activated photosensitizer leads to the formation of reactive oxygen species, which will damage tumor or normal cells located at the site of its production (6,7). PDT is FDA approved for superficial and obstructing non-small cell lung cancer, obstructing esophageal cancer, and Barrett's esophagus with high-grade dysplasia. It has been investigated for treatment of locally recurrent prostate cancer following radiation therapy (2,6,8-12), and trials of Tookad-mediated PDT for prostate cancer are currently open in Canada¹ and in the United Kingdom². One of the largest advantages that PDT offers to the treatment of prostate cancer is that it can typically be used to treat tissues already exposed to a maximal dose of RT (10). Moreover, PDT itself can be repeated several times if necessary (10).

In our recently closed Phase I clinical trial of motexafin lutetium (MLu) PDT for prostate cancer, we treated patients (n=17) who experienced locally recurrent disease after radiation therapy (13). Patients were treated with increasing doses of MLu (0.5–2 mg/kg), decreasing time interval to light delivery (3-24 hrs), and increasing light fluence (25-150 J/cm²). Light was delivered to a prescribed dose that was directly measured within the gland by interstitially inserted isotropic detectors. Illumination of the entire prostate was accomplished by using a brachytherapy template to position the interstitially-placed treatment fibers 1 cm apart. This distance was chosen based on studies of canine prostate, which found fiber spacing at distances of 1 cm to provide effective and safe light distribution (13,14).

The primary findings of the MLu-PDT prostate trial have included reports of considerable intra-patient and inter-patient heterogeneity in light distribution, photosensitizer uptake, oxygenation, and tissue optical properties within the prostate (15,16). Although these results are not dissimilar from that found in other studies of PDT (15-19), this heterogeneity makes treatment dosimetry challenging. Integrated treatment dosimetry that utilizes real time measurement of fluence rate and tissue optical properties, together with photosensitizer concentration, oxygenation and/or blood flow, has been proposed as a method toward optimizing PDT (9,12). In addition to these methods for physical dose or efficacy monitoring, the rapid assessment of biological response could significantly improve patient care by providing a means for the early identification of those who would benefit from additional treatment sessions. In the treatment of prostate cancer, PSA response may provide a simple, inexpensive monitoring tool with the potential to rapidly report on treatment effect, thereby facilitating the identification of patients who should be further considered for subsequent PDT treatment sessions.

¹ClinicalTrials.gov. Accessed on December 11, 2007.

²www.prostatepdt.com. Accessed on December 11, 2007.

Short-term increases in serum PSA levels result from manipulation or treatment of the prostate, through processes such as digital rectal examination (DRE), cystoscopy, prostate biopsy, transurethral resection of the prostate (TURP), cryotherapy, electrovaporization, or other procedures. (20-26). The rise in PSA correlates to the invasiveness of procedure (20), and elevated PSA levels are generally detected over a period ranging from less than 1 h to several weeks after the procedure (21-23,25). The biochemical success of prostate treatment is typically measured through the extent of PSA reduction subsequent to its initial therapy-induced spike (22,25). Yet in laser prostatectomy, peak PSA values at 24 h after the procedure were shown to correlate with the dose of energy delivered (26). Therefore, early PSA response may also be a useful indicator of treatment effect.

The objective of this study was to determine the time course of serum PSA response to MLu-PDT of prostate cancer. The relationship between delivered PDT dose and PSA response was evaluated. PSA response was also considered as a function of whether or not PDT led to a decrease in prostate photosensitizer concentration during illumination, which for some photosensitizers can indicate the presence of photobleaching and a photochemical effect (27).

MATERIALS AND METHODS

Patient Selection

Eighteen patients were enrolled on a phase I clinical trial of interstitial MLu-mediated PDT for prostate cancer at the University of Pennsylvania. Seventeen patients went on to receive MLu and interstitial light (PDT) and these 17 patients are considered for the present analysis. The details of patient characteristics and the clinical trial were previously reported (13,15,28) and are summarized here. Data on MLu tissue concentration have been previously presented (13), as has serum PSA levels for one patient (13), but in the present report we uniquely consider these data in relation to each other. The Phase I clinical trial was performed according to protocol approved by the Institutional Review board of the University of Pennsylvania, the Clinical Trials and Scientific Monitoring Committee (CTSRMC) of the University of Pennsylvania Cancer Center, and the Cancer Therapy Evaluation Program (CTEP) of the National Cancer Institute. The study of MLu-mediated PDT was conducted on patients with biopsy-proven locally recurrent prostate carcinoma following radiation therapy. Each patient signed the informed consent and underwent a history and physical, MRI of the prostate, bone scan, urological evaluation, and laboratory studies including complete blood count, biochemistry profile, lipid panel and prostate specific antigen (PSA) levels. Seventeen patients were treated, eight with recurrence following external beam radiation therapy and nine following brachytherapy. Exclusion criteria included any evidence of distant metastasis or extracapsular invasion, primary T3 or T4 tumors, prostate volume >50 cc, PSA >20 ng/mL, history of Grade III or IV genitourinary or gastrointestinal toxicity with previous therapy, and inadequate renal, hepatic or hematological function. One patient was found to have extracapsular extension of disease after being enrolled on the protocol and did not receive PDT.

Prostate PDT

PDT was administered as reported earlier (13,15,28). Briefly, using images collected by transrectal ultrasound at two weeks prior to PDT, a treatment plan was developed in Multimedia Medical System software for interstitial fiber placement. Cylindrical diffusing fibers of varying lengths were used as light sources, spaced 1 cm apart at positions determined by the treatment plan in order to cover the entire length of the prostate at a particular position. Within 24 hours prior to light delivery, 0.5–2 mg/kg motexafin lutetium (MLu; CTEP-NCI) was administered intravenously, over 5–10 minutes, as a sterile, pyrogen-free 2 mM (2.3 mg/ml) solution in 5% mannitol/water (see dose escalation in Table 1). After an interval of 3–24 h, 732-nm light was delivered (Laser Model 730, 15-W diode, Diomed, Ltd., Cambridge, UK)

at a fluence rate of 150 mW/cm, with light fluence from 25 to 150 J/cm² (see Table 1). PDT was performed in a surgical suite with precautions to prevent unplanned photosensitizer activation, including filtered operating room lighting and covering of the patient's exposed skin.

MLu Measurement

Under ultrasound guidance needle biopsies were collected from each prostate quadrant at times both before and after light administration. Biopsy specimens were frozen on dry ice, protected from light, and transported to the laboratory for storage at -80°C . At time of analysis, biopsy tissue was thawed and multiple biopsy samples were combined as needed to obtain a total of 5-25 mg. Only biopsies collected at the same time point, *i.e.* either pre- or post-PDT, were combined. If more than one appropriately-sized sample was available for analysis then the resulting data were averaged to provide a single photosensitizer value for the tissue of each patient at each timepoint. Specimens were placed in a 2 mL capped polypropylene tube and homogenized (Polytron 1200) in 400 μL of phosphate buffer (24 mM, pH 7.5). Homogenates were mixed with 400 μL of chloroform, then 400 μL of methanol was added. After centrifugation (3500 rpm, 15 min) the organic layer was collected and 200 μL was transferred to a cuvette. The fluorescence of the homogenized sample was measured by a spectrofluorometer (FluoroMax-3, Jobin Yvon, Inc., Edison, NJ) with λ_{ex} of 474 nm and λ_{em} of 740 nm (emission scan range from 650 to 850 nm). MLu concentration in the tissue was calculated based on the change in fluorescence resulting from addition of a known amount of MLu to each sample after its initial reading. Data are presented as nanograms of MLu per milligram of tissue (16). The effect of PDT on tissue photosensitizer level was calculated from these data as the ratio of post-PDT to pre-PDT tissue photosensitizer concentration.

PSA Measurement

Baseline PSA was defined as the serum PSA value closest to the time of PDT within ~ 2 months prior to treatment. Post-procedure serum PSA was drawn at one day after completion of PDT and analyzed at Hospital of University of Pennsylvania using standard procedure. Subsequent PSA levels were drawn at each follow up visit, which by protocol were scheduled at 2 weeks after discharge, monthly for 3 months, then every 3 months for up to two years. These visits actually provided PSA values for the following time ranges after PDT: day 1, weeks 1-3 (day 4-24), month 1 (day 26-55), month 2 (day 60-95), month 3 (day 102-126), months 4-6 (day 129-206), months 7-11 (days 211-297). A small amount of leeway was allowed in the actual number of days encompassed by each protocol-defined follow-up interval in order to ensure that sequential measurements on patients were placed in their intended time course. If a patient had more than one PSA measurement within the indicated time frame then the reported value is the average of all collected samples. Percent change in PSA was defined as $[(\text{post-PDT} - \text{pre-PDT})/\text{pre-PDT}] * 100\%$, using the pre-PDT PSA value closest to the start of PDT treatment.

Statistical Analysis

Continuous outcomes were characterized by descriptive statistics, *e.g.*, the mean, median, standard error, and range. Inter-patient variability was evaluated by the coefficient of variation, calculated as the $[(\text{standard deviation}/\text{mean}) * 100]$. Patients were dichotomized into low and high PDT dose groups based on values less than and equal to or greater than the median delivered PDT dose, respectively. The nonparametric Wilcoxon signed ranks test was used to compare pre- and post-PDT PSA level within patients. The biochemical delay was defined as the time from PDT to a nonreversible increase in PSA to a level equal to or greater than baseline. In other words, the PDT-induced increase in PSA was excluded from this analysis since it was a temporary, reversible phenomenon, and data were analyzed for a rise in PSA after their post-

therapy minimum. Biochemical delay was estimated by the Kaplan-Meier method and group comparisons were assessed by the log rank test. Patients who did not experience this event were censored on the date of their final PSA measurement within the time frame of this study (up to 297 days after PDT). The coefficient of determination (R^2) was used to assess the magnitude of the correlation between tissue photosensitizer concentration and the percent change in PSA at 24 h after PDT. All p values are 2-sided. All analyses were conducted with SPSS 14 (SPSS, Inc., Chicago, IL) or JMP 5.1 (SAS Institute, Cary, NC).

RESULTS

Seventeen patients were treated on a phase I trial of MLu-PDT for cancer of the prostate. While toxicity of treatment was a primary study endpoint, data on serum PSA and tumor photosensitizer uptake were collected before and after light treatment. Table 1 summarizes the PDT conditions and early PSA response of these patients. Photosensitizer doses of 0.5 mg/kg (n=3 patients), 1.0 mg/kg (n=4), or 2 mg/kg (n=10) were employed in combination with drug-light intervals of 3, 6 or 24 h. Pre-PDT prostate photosensitizer levels (mean $\pm$ SE) corresponding to these injected doses were 0.35 ± 0.04 ng/mg at 0.5 mg/kg (24 h, n=3); 0.65 ± 0.07 ng/mg at 1 mg/kg, 24 h (n=3); 0.47 ng/mg at 1 mg/kg, 6 h (n=1); 3.38 ± 0.74 ng/mg at 2 mg/kg, 6 h (n=3); and 4.99 ± 0.90 ng/mg at 2 mg/kg, 3 h (n=6). All patients who received low drug doses (0.5 or 1 mg/kg) were treated with low fluence (25 J/cm^2), whereas patients who received a 2 mg/kg dose were treated at fluences of 25 (n=2), 50 (n=2), 100 (n=4) or 150 J/cm^2 (n=2). This lead to delivered PDT doses (product of photosensitizer level and fluence)

less than $20 \frac{\text{ng} \cdot \text{J}}{\text{mg} \cdot \text{cm}^2}$ for patients treated with low (0.5 or 1.0 mg/mg MLu) photosensitizer doses, while patients who received 2 mg/kg MLu experienced delivered PDT doses of 76 to

$819 \frac{\text{ng} \cdot \text{J}}{\text{mg} \cdot \text{cm}^2}$. The median delivered PDT dose was $116 \frac{\text{ng} \cdot \text{J}}{\text{mg} \cdot \text{cm}^2}$.

Fourteen of the 17 treated patients were PSA-evaluable, meaning that PSA levels were detectable in the pre-PDT samples. Among these 14 patients, pre-PDT serum PSA values ranged from 1.8 ng/ml to 15.4 ng/ml (median 6.4 ng/ml). PSA levels could be expected to exhibit two types of changes after PDT: 1) an increase in PSA levels shortly after PDT could result from PDT insult on the prostatic tissue and 2) a decline in PSA levels at times more removed from treatment could result from a reduction in tumor burden. Figure 1A displays the time course of the change in serum PSA levels in a patient treated with high dose PDT (150 J/cm^2 , 2 mg/kg MLu, 3 h drug-light interval). PDT was immediately followed by a large increase in PSA, which rapidly dropped to levels below the baseline measurement. Among all of the patients, PSA values generally reached their maximum within 1 day to 3 weeks after PDT, although one patient (#7) experienced a peak at his one month follow-up and one patient (#10) did not experience an increase in PSA levels within the short term after PDT.

To summarize the effect of PDT on PSA, the change in PSA was calculated for each patient in multiple post-PDT time frames: day 1, weeks 1 – 3, month 1, month 2, month 3, months 4-6 and months 7-11 (Figure 1B). On average (mean $\pm$ SE) serum PSA levels significantly increased by $98 \pm 36\%$ within 1 day after PDT ($p=0.007$), and remained elevated ($53 \pm 37\%$) through the following 1-3 weeks. However, there was substantial inter-patient variability in percent change in PSA at both time intervals, as indicated by coefficient of variation values of 127% and 253% at day 1 and week 1-3, respectively. On average, within 26-55 days (month 1) after PDT serum PSA levels dramatically declined to a level indistinguishable from baseline (mean $\pm$ SE, $1 \pm 10\%$). This decline was followed by a steady increase in PSA levels as disease progressed, and PSA levels significantly exceeded their pre-PDT values within 129-206 days (months 4-6) after treatment. Overall, these data demonstrate that early and substantial

increases in serum PSA levels may be one of the first indications of treatment effect in interstitial PDT of prostate cancer.

Strong temporal changes in PSA response to PDT are apparent in the data of Figure 1B regardless of the fact that these responses are averaged over patients who experienced a very broad range of PDT doses (see Table 1). To more closely examine the effect of PDT dose on PSA response, patients were divided into approximately equal sized groups, defined as those

who received PDT doses less than the median value of $116 \frac{\text{ng} \cdot \text{J}}{\text{mg} \cdot \text{cm}^2}$ vs. those who received PDT doses equal to or greater than this value. Figure 2 demonstrates striking differences in the first month of PSA responses in patients who received high vs low PDT dose. The percent increase (mean $\pm$ SE) in PSA at day 1 was greater in patients who received high ($119 \pm 52\%$) than low ($54 \pm 27\%$) PDT dose, with only the high dose patients experiencing an increase in serum PSA levels over baseline that closely approached statistical significance ($p=0.055$). Furthermore, at their one month follow-up, high PDT dose patients trended toward a decrease in PSA levels relative to their baseline ($-11 \pm 15\%$), whereas low PDT dose patients continued to exhibit PSA levels that on average were slightly higher than baseline ($15 \pm 10\%$). Differences in PSA response as a function of PDT dose disappeared by the conclusion of 2 months after PDT, with both groups of patients demonstrating a $\sim 10\%$ increase in PSA levels.

The duration of biochemical delay, the length of time between PDT and a nonreversible increase in PSA to a value equal to or greater than baseline was calculated for the high and low PDT dose groups. It should be noted that: 1) baseline PSA level was similar in patients treated at high vs. low PDT dose (mean $\pm$ SE, 6.5 ± 1.5 vs. 8.2 ± 1.5 ng/ml, respectively) and 2) time to the minimum PSA value after PDT was also similar in high and low PDT dose patients (mean $\pm$ SE, 33 ± 7 vs. 45 ± 9 days, respectively). In contrast, the duration of biochemical delay was longer for the high PDT dose group as compared to the low PDT dose group (median 82 days vs. 43 days, respectively, $p=0.024$, Figure 3). Between 19 and 160 days after PDT, all 6 patients in the low PDT dose group experienced a PSA value greater than or equal to baseline. The patient whose biochemical delay was noted to be 160 days did not adhere to the follow-up schedule and a shorter delay is quite likely. In contrast, 3 patients in the high dose group failed to reach their respective pre-PDT PSA levels in follow-ups that extended through 123 - 283 days. However, while the high dose PDT patients did demonstrate a significantly greater biochemical delay, all patients did ultimately experience biochemical failure, as defined by an increase in PSA to a value at least 0.5 ng/ml greater than the minimum post-PDT value in three consecutive follow-up visits. Substantial variability in time to biochemical failure existed among patients, which masked any difference between the high and low dose groups.

Due to the design of this clinical trial, high PDT doses generally incorporated both high drug dose and high light fluence, while low PDT doses generally incorporated both low drug dose and low light fluence (see Table 1). Therefore, the increase in PSA levels at high PDT doses could, in fact, be driven by an association between PSA level and either drug dose or fluence individually. We explored these possibilities, finding no association between PSA and the fluence of light delivery (data not shown). There did appear to be a weak positive correlation between percent change in PSA at 1 day after PDT and the pre-PDT tissue MLu concentration (Figure 4A, $R^2 = 0.29$, $p=0.09$), which emphasizes that the effects photosensitizer content may dominant over effects of light dose in the relationship between PDT dose and PSA response under the conditions studied.

If pre-PDT prostate photosensitizer concentration is dominant in the effect of PDT dose on PSA response, then it also becomes relevant to examine how changes in prostate photosensitizer concentration during PDT are related to PSA response. Change in MLu concentration during PDT was calculated as the ratio of post-PDT to pre-PDT tissue

concentration. A decrease in M_{Lu} was detected in 11 of 16 evaluable patients, whereas an increase in M_{Lu} concentration was found in 5 patients. Figure 4B summarizes the change in prostate M_{Lu} concentration in each patient as a function of injected dose.

The relationship between the post-PDT/pre-PDT tissue concentration and the maximum PDT-induced increase in PSA was considered for 12 patients who exhibited a short-term increase in PSA after PDT. Patients were divided into two groups, those who demonstrated a decrease in drug level with PDT and those who demonstrated an increase. Among those patients who experienced a decrease in prostate photosensitizer concentration during PDT, the average ($\pm$ SE) maximum change in PSA was $136 \pm 60\%$ ($p=0.008$), while the maximum change was only modest ($52 \pm 23\%$) among patients who did not demonstrate a decrease in prostate M_{Lu} concentration (Figure 5).

DISCUSSION

Treatment options for locally recurrent prostate cancer after radiation therapy are limited. PDT is an attractive experimental modality because it offers the possibility of repeat treatments and minimal morbidity (10). Therefore, a means for early assessment of patient response to prostate PDT would be a valuable tool in planning patient care. Other modalities that treat the prostate can result in large, but transient increases in serum PSA levels (22,25). Furthermore, these elevated PSA levels were found to correlate with treatment dose (energy) in laser-mediated electrovaporization of the prostate (26). In the present study we report that 1) M_{Lu}-PDT of human prostate cancer results in a significant short-term increase in serum PSA levels and 2) the change in PSA is related to PDT dose, as well as to PDT effect on photosensitizer concentration in the treated tissue.

Several clinical studies of PDT for prostate have been reported, including treatments that utilized the drugs hematoporphyrin derivative, aminolevulinic acid, meso-tetrahydroxyphenyl chlorin (mTHPC), and Tookad (2,6,10). Although some reports do document PSA response at roughly one month after PDT (8,11,29) to our knowledge the present study is the first to evaluate PSA within one day to several weeks of treatment. A short term increase in PSA after treatment of the prostate can be caused by cellular damage, leading to the release of PSA into the circulation (25). We found such an increase after M_{Lu}-PDT despite the limitation that damage was not sufficient to control disease progression, i.e. ultimately all patients experienced biochemical failure. Nevertheless, a ~ 2 fold difference in PSA response at 24 h after PDT was noted in patients treated at high vs. low PDT dose, which suggests that higher, more effective PDT doses will lead to even greater PSA release. In some instances a time lapse of 2 months occurred between baseline PSA measurement and PDT, thus disease progression over time may contribute to the increase in PSA after PDT. However, the average ($\pm$ SE) absolute change in PSA at 1 day after PDT (6.1 ± 2.7 ng/ml) greatly exceeded the increase in PSA (1.8 ± 0.4 ng/ml) over an average ($\pm$ SE) of 160 ± 33 days immediately preceding baseline measurement in patients ($n=13$) who were not on hormone therapy prior to the baseline blood draw. These data strongly suggests that increases in PSA after PDT were predominantly a result of treatment, with less effect from disease progression in a potential ~ 60 day window. PSA values (average $\pm$ SE) after PDT increased to 13.2 ± 3.3 ng/ml (range 4.4 – 41.2 ng/ml) on day 1 and 9.7 ± 2.5 ng/ml (range 2.3 – 37.9 ng/ml) in weeks 1-3. Since these levels are far below those reported after prostate cryosurgery (average maximum of 155 ng/ml; range 18.9 – 490.5 ng/ml) (25), it is likely that more effective PDT protocols will lead to greater PSA release.

The magnitude of increases in serum PSA after therapy necessarily will be a function of not only the severity of prostate damage, but also the presence of functional vasculature to deliver PSA to the systemic circulation. Therefore, specific trends in PSA response to PDT may differ among photosensitizers and protocols that alter the balance of direct cell kill vs. vascular

damage. For example, pre-clinical investigations in subcutaneous human prostate tumor xenografts found Tookad PDT to lead to immediate (within 7 h) declines in PSA levels (30). This response could be a result of the rapid vascular shutdown found during Tookad PDT, which is a purely vascular treatment (31). It is also likely a function of the animal model studied, and PSA levels in humans cannot be expected to change as rapidly since PSA half life is longer in humans (30). Nevertheless, the present study found significant PDT-induced increases in PSA following MLu-PDT conditions that are considered to be predominantly vascular targeting, i.e. a short drug-light interval. Therefore, it is not unreasonable to expect that PDT with more cellular-acting drugs such as ALA and mTHPC will also lead to PSA increases.

Since prostatic manipulation, including biopsy collection (24), can lead to release of PSA, there is reason to believe that the process of interstitial fiber placement for PDT will cause a spike in serum PSA. However, in the present report all patients experienced the same process for interstitial placement of the treatment fibers so this factor cannot explain the difference in PSA response as a function of PDT dose. On the other hand, patients treated at higher PDT dose also had slightly longer treatment times and theoretically prolonged procedural trauma. The possible effect of treatment time on PSA response can be assessed from the relationship between treatment fluence, *i.e.* treatment time, and PDT-induced change in PSA levels. No association between fluence and PSA response was found, suggesting that the duration of interstitial probe placement did not affect PSA response over the range of exposure times used in this trial.

Subsequent to treatment-induced increases in PSA, its serum levels will decline if therapy was sufficient to reduce disease burden and prostate volume, thereby decreasing the number of cells releasing PSA (11,24). A correlation between reduction in prostate volume and decreasing serum PSA levels has been found at 6 weeks after electrovaporization therapy (22), and a correlation between the extent of prostatic necrosis and the percent change in PSA was found at 4 and 12 weeks after PDT (32). In our patients, PSA response at one month after PDT is similar to that which has been found in other PDT trials: some patients experienced a reduction in PSA levels to below baseline, but generally this was followed by steadily increasing levels (8,11). However, a PSA response lasting up to 6 months after PDT (the length of the study) was reported by Trachtenberg et al (29) in the majority of men who experienced a necrosis volume greater than 20% after localized illumination of the prostate with Tookad-mediated PDT.

Overall, the findings of this report show higher PDT dose to lead to a substantially greater increase in PSA at 24 h after PDT (119% vs. 54%). These data suggest that higher PDT dose creates greater tissue damage, which is reported by a larger initial spike in PSA. The biological significance of these findings is emphasized by a longer delay (82 vs. 43 days) between the time of PDT and a nonreversible return to baseline PSA level in patients who received high vs. low dose treatment. These data suggest that PSA change after PDT can provide information about tissue effects of treatment. Thus, early post-treatment PSA levels, if confirmed in larger studies to correlate with tissue response, can provide opportunity to identify patients who should be more carefully considered, e.g through prostate biopsy, for multi-session PDT treatment protocols. Even in the absence of multi-session PDT, the early PSA response may help identify patients who should have a repeat biopsy early, due to high suspicion of inadequate treatment.

Calculation of PDT dose includes the pre-PDT photosensitizer level, which, as an independent variable, appears to be weakly correlated with PDT effect on PSA. Higher pre-PDT photosensitizer levels were associated with a larger post-PDT increase in serum PSA, suggesting that greater tissue damage occurred in patients who had higher prostate MLu concentration at the time of illumination. In biopsies collected immediately prior to PDT, MLu

concentration in prostate tissue generally increased with increasing injected dose and decreasing drug-light interval but, consistent with previous reports, there was considerable variability in tissue MLu levels (15,16). Inherent differences in drug concentration in cells throughout the prostate likely contribute greatly to this variability. Furthermore, the biopsies were taken independent of knowledge on the location of disease foci and could include both normal as well as cancerous tissue. A more rigorous investigation of the relationship between photosensitizer content and PSA response will be possible in trials that enroll a larger number of patients at a fixed light dose.

After PDT, a decrease in MLu fluorescence is evident in 11 of 16 patients who had tissue samples available for analysis. However, again variability exists due to inherent differences in drug levels that are averaged over tissue type (normal vs. cancerous) and location. The extent of PDT effect on photosensitizer concentration may also be affected by reaccumulation of photosensitizer in tissue during treatment (33). This factor may be particularly important when plasma levels of photosensitizer are high, such as at short drug-light intervals, and when the duration of illumination is long, i.e. high light doses (33). In general, decreases in tissue photosensitizer concentration during PDT are attributed to photobleaching, however little has been done to characterize photobleaching during MLu-mediated PDT.

Several reports document the presence of a correlation between PDT-created tissue damage and dosimetric measures that incorporate photosensitizer photobleaching (27,33-36). In this study, the presence of a decrease in prostate MLu concentration during PDT was associated with larger post-PDT increases in PSA levels. PSA levels changed by a maximum of 136% vs. 52% in patients who did or didn't demonstrate a decrease in prostate photosensitizer concentration, respectively. These results suggest that photobleaching during MLu PDT reports tissue damage, which is reflected by increases in serum PSA, but caution must be exercised in interpreting these data due to small sample sizes, the presence of substantial heterogeneity in prostate photosensitizer concentration, and the generally limited availability of information on the mechanisms and photochemical basis of MLu photobleaching. Nevertheless, these data do provide strong rationale to justify the incorporation of measures of photosensitizer photobleaching in clinical trials of PDT, especially under conditions where accompanying comprehensive measurement of tissue response is feasible.

In conclusion, photodynamic therapy is under evaluation for the treatment of locally recurrent prostate cancer after radiation therapy. PDT provides minimally invasive treatment with few adverse effects, and further studies are warranted to evaluate efficacy and improve therapy delivery. There is considerable intra- and inter- patient variability in all aspects of PDT dosimetry including drug concentration, fluence rate, and optical properties. Thus the ability to perform individualized, *in situ* measurement of PDT dose is valuable, as would be a means for the rapid individualized assessment of PDT effect. The data of the present report strongly promote the need for individualized measures of PDT delivery, *e.g.* that reported by *in vivo* light dosimetry, and individualized measures of PDT effect, *e.g.* that reported by serum PSA levels, to be incorporated into clinical trials of prostate cancer.

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Figure 1.

Time course of PSA response to PDT in patient 17 (A) and of the patient -averaged (mean $\pm$ SE) percent change in PSA after PDT (B). The % change in PSA was calculated as $100 \times (\text{PSA}_{\text{after PDT}} - \text{PSA}_{\text{before PDT}}) / \text{PSA}_{\text{before PDT}}$ for each patient using the pre-PDT PSA value closest to the time of treatment and the post-PDT PSA value in the time frame of interest. In plot A, the dashed line indicates when PDT was performed and the numerical insets indicate baseline, post-PDT maximum, and post-PDT minimum PSA values. In plot B, $n = 12, 13, 13, 11, 9, 10,$ and 7 for each respective timeframe, in increasing order. Wk = week; m=month; “*” = $p < 0.05$ for Wilcoxon signed rank test comparing post-PDT PSA values to baseline measurement in the same patient.

Figure 2.

The average (mean $\pm$ SE) percent change in PSA a function of time after PDT in patients who

experienced a PDT dose $<$ (open bars) or $\geq$ (closed bars) the median dose of $116 \frac{\text{ng} \cdot \text{J}}{\text{mg} \cdot \text{cm}^2}$. PDT dose was calculated as the product of tissue photosensitizer concentration and light dose, $n = 4-6$ (open bars) or $7-8$ (closed bars). “+” = $p < 0.06$ for Wilcoxon signed rank test comparing post-PDT PSA values to baseline measurement in the same patient.

Figure 3.

Kaplan-Meier estimation of biochemical delay in the PSA response in patients treated with a

PDT dose $\leq$ or $\geq$ the median dose of $116 \frac{\text{ng} \cdot \text{J}}{\text{mg} \cdot \text{cm}^2}$. Duration of biochemical delay was defined as the length of time between PDT and a nonreversible (*i.e.* not PDT-induced) increase in PSA to a value $\geq$ baseline. N = 6 and 8 for low and high dose PDT, respectively.

Figure 4.

The PDT-induced change in PSA at 24 h after illumination as a function of pre-PDT photosensitizer concentration in the prostate (A); $R^2 = 0.29$, $p = 0.09$. MLu concentration in prostatic biopsies collected before (closed symbols) and after (open symbols) PDT (B); times indicate the interval between drug administration and light delivery. MLu concentration was determined by spectrofluorometric assay of drug levels in prostate biopsies.

Figure 5.

The average (mean $\pm$ SE) maximum percent change in PSA in patients (N=8) who experienced a decrease in prostate MLu concentration during PDT (MLu post/MLu pre <1) vs. those (N=4) who did not experience a decrease in prostate MLu concentration (MLu post/pre >1).

Maximum effect on PSA was calculated as $100 \times (\text{PSA}_{\text{after PDT}} - \text{PSA}_{\text{before PDT}}) / \text{PSA}_{\text{before PDT}}$ using the pre-PDT PSA value closest to the time of treatment and the post-PDT PSA value with the largest increase within 1 month of PDT. “*” = $p < 0.05$ for a Wilcoxon signed rank test comparing post-PDT PSA values to baseline measurement in the same patient.

Table 1
Treatment parameters and early PSA response in patients receiving MLu-PDT of prostate cancer

Patient #	MLu dose (mg/kg)	Drug-light interval (h)	Fluence (J/cm ²)	Pre-PDT MLu (ng/mg)	PDT dose (ng/mg * J/cm ²)	PSA (ng/ml)			
						pre-PDT	day 1 post-PDT	weeks 1-3 post-PDT	month 1 post-PDT
1	0.5	24	25	0.28	7	ND	---	---	---
2	0.5	24	25	0.34	9	10	NA	12	10
3	0.5	24	25	0.42	11	12	12.3	NA	10.2
4	1	24	25	0.77	19	12.1	14.4	10.8	14.1
5	1	24	25	0.65	16	6.4	11.4	8.3	6.5
6	1	24	25	0.52	13	3.4	7.3	4.5	4.9
7	1	6	25	0.47	12	5.1	NA	5.8	7.2
8	2	6	25	3.03	76	ND	---	---	---
9	2	6	50	2.32	116	5.9	4.4	6.3	5.3
10	2	6	100	4.8	480	6.7	5.6	5.7	5.7
11	2	3	25	4.65	116	1.8	6.4	2.3	---
12	2	3	50	3.55	177	8.4	11.9	8.9	6.7
13	2	3	100	NA	---	3.4	7	5.5	4.7
14	2	3	100	6.73	673	4	5.2	6.1	5.4
15	2	3	150	1.71	257	ND	---	---	---
16	2	3	100	7.83	783	6.4	31.7	37.9	5.1
17	2	3	150	5.46	819	15.4	41.2	11.4	2.7

ND= not detectable

NA= not available

* Although insufficient sample size prevented *ex vivo* measurement of prostate MLu concentration in patient 13, drug concentration measured by absorption spectroscopy, a technique that has been validated relative to the *ex vivo* spectrofluorometric measurements in these patients (16), indicates that patient 13 experienced a PDT dose that was ≥ 140 ng/mg * J/cm² (15).