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Effects of Photodynamic Therapy on Peripheral Nerve: *In Situ* Compound-Action Potentials Study in a Canine Model

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Abstract

Objective: Our aim is to investigate the effects of photodynamic therapy (PDT) on peripheral nerve conductivity. **Background Data:** Interstitial PDT has been demonstrated as a promising treatment modality for prostate cancer. However, the sensitivity of nerves, in the immediate vicinity of the prostate gland, to PDT procedures has not been studied. This study attempts to establish an *in situ* canine model to evaluate direct PDT effect on peripheral nerves. **Methods:** PDT was performed by irradiating the cutaneous branches of the saphenous nerve at 763 nm with light doses of 50-200 J/cm² after i.v. infusion of the photosensitizer Tookad (0-2 mg/kg). Evoked compound-action potentials (CAP) were recorded directly from the surface of the saphenous nerve. The latencies to onset and conduction velocities were determined during PDT and 1-week post-PDT. **Results:** Nerve and surrounding tissue damage corresponded well with drug/light doses. With Tookad doses of 2 mg/kg, treatment with 50 J/cm² induced little change in saphenous nerve conduction properties. However, treatment with 100 J/cm² resulted in localized nerve injury and decreases in nerve conduction velocities, and treatment with 200 J/cm² severely damaged the nerve. **Conclusions:** This canine model adequately demonstrates effects of Tookad PDT on peripheral nerves. Direct irradiation of 100-200 J/cm² can alter nerve conduction and induce nerve damage. Therefore, possible side effects of interstitial PDT on the pelvic plexus need to be investigated in future studies.

INTRODUCTION

PHOTODYNAMIC THERAPY (PDT) is becoming an accepted treatment modality for malignant and nonmalignant diseases. Although the majority of uses treat superficial lesions, interstitial ablation of solid tumor such as prostate cancer is now being investigated. The feasibility of using interstitial PDT for prostate cancer treatment has been evaluated in various animal models by this and other groups.¹⁻⁹ Recent clinical trials demonstrate that PDT is a promising means for prostate cancer treatment.^{10,11} Therefore, it becomes more important that the possible side effects of interstitial PDT on adjacent structures of the prostate gland be further investigated in order to preserve these structures while still achieving total ablation of prostate cancer. Nerve damage and subsequent erectile dysfunction and incontinence disorder have been reported in radiation therapy and radical prostatectomy. Knowledge of the anatomy of nerves adjacent to the prostate gland and innervation of the prostate gland has improved the nerve preservation of these conventional treatments.^{12,13} However, effects of PDT on the prostate-related nerve

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system have not been fully explored. This study attempts to establish an *in situ* canine model to evaluate direct PDT effects on peripheral nerve tissue.

The ultimate goal of this study is to correlate photodynamic lesions to the function and structure of the pelvic plexus. Considering the complexity and limited accessibility of the pelvic plexus, the initial pilot study used the cutaneous branches of the canine saphenous nerve for studying the effects of PDT on the nerve conductivity. Evoking and recording compound-action potentials (CAP) in the distal portion of the saphenous nerve *in Situ*¹⁴ was used to investigate the effect of Tookad-mediated PDT on the peripheral nerve conductivity. The photosensitizer Tookad is a pure palladium substituted bacteriochlorophyll derivative with a maximum absorption wavelength of 763 nm and a fast clearance rate. Its effectiveness on ablating prostate gland and prostate cancer has been demonstrated in various animal models.^{2,3,15} The preliminary information obtained from this conductivity study will then be used to determine the dose levels for direct pelvic nerve irradiation in future work. This is the first report on the pathological nature of PDT affecting the peripheral nerve tissue in a canine model.

MATERIALS AND METHODS

Saphenous nerve conductivity experiments were performed in healthy adult male beagle dogs ($n=9$) according to a protocol approved by the Institutional Animal Care and Use Committee of HealthONE Alliance.

Evoking and recording compound-action potentials (CAP)

The cutaneous branch of the saphenous nerve (right pelvic limb) was exposed through an incision where it accompanied the cranial branch of the saphenous artery. Stimulating electrodes were placed proximal to the recording electrodes. The distance between the stimulating and recording electrodes was approximately 6 cm with the ground electrode placed between the recording and stimulating sites (Fig. 1). Stimulating and recording electrodes were custom-built from a small section of plastic tubing and small gauge insulated silver wire. The tubing (~1 cm long) was cut lengthwise to allow it to wrap around the nerve, ensuring direct contact between the bared electrode tip and the nerve surface. The distance between wires in an electrode pair was 10 mm. Tissue temperature was measured in the center of the incision site adjacent to the nerve using a thermometer (Digisense, model 8528-20, Ayer Rajah Crescent, Singapore). The core body temperature was monitored by a rectal probe and maintained at around 37°C.

Rectangular pulses were delivered by a stimulator (Grass Instruments, model S44, West Warwick, RI) through a stimulation isolation unit (Grass Instruments, model SIU5A) and a constant current unit (Grass Instruments, model CCU1A) at a rate of 4 pps, with a 5- μ s duration. Each stimulus-evoked action potential was led into a preamplifier (Grass Instruments, model P511K), usually with an amplification factor of 100,000. Each stimulus-evoked compound action potential was detected by a high-impedance probe (Grass Instruments, model HIP5) and fed into a differential amplifier (Grass Instruments, model 511K), with a band pass of 10-3000 Hz (-3 dB). The amplified signals and stimulus-synchronization pulses were led into a signal-averaging, digital oscilloscope (Hewlett Packard, model 54501A, Melrose, MA) and into a 16-bit analog-to-digital converter (National Instruments, Austin, TX). Each averaged evoked potential was produced by averaging 64 successive responses to nerve stimulation and contained a minimum of 1500 data points with a sampling period of 5 μ s/point. The averaged CAPs were analyzed using LabVIEW (National Instruments, version 7). The amount of current needed to produce threshold responses and maximum amplitudes of the evoked CAP were determined. Latencies were measured from the onset of the stimulus artifact to the initial positive (downward) peak of the averaged CAP.¹⁴ Conduction velocities were determined prior to PDT, during PDT, immediately post-PDT, and 1 week post-PDT. Control studies

(surgical control and light control) were performed on the contralateral saphenous nerve (left pelvic limb) postoperatively and at a 1-week follow up.

PDT procedures

The photosensitizer drug Tookad (Pd-bacteriopheophorbide, Steba Biotech, Toussus-Le-Noble, France) was administered by slow i.v. infusion over a period of 10 min (0 mg/kg, $n = 1$; 1.0 mg/kg, $n = 5$; 2.0 mg/kg, $n = 3$). All PDT treatments were performed superficially with a microlens fiber (Medlight S.A., Mullhouse, Switzerland) on the surface of the saphenous nerve at a light dose of 50, 100, or 200 J/cm². The duration of light irradiation was 5.5 min for 50 J, 11.1 min for 100 J, or 22.2 min for 200 J, respectively. The light source was a 763-nm diode laser (Ceralas; CeramOptec GmbH of Biolitec AG, Bonn, Germany). The onset of drug infusion was recorded as time 0. Light irradiation of 150 mW/cm² started at 7.2 min for 50 J, 4.5 min for 100 J, or 0 min for 200 J after the onset of drug infusion so the drug infusion and light irradiation overlapped in a “peak mode”. In this peak mode, i.e., overlapping of the time point of the maximal plasma drug concentration ($C_{\max}$) and mid-point of light irradiation, it is now known to center the light irradiation period around the single peak of the photoactive monomeric photosensitizer concentration in blood and hence, putatively, should be maximally effective. Because of the close proximity of the saphenous artery to the saphenous nerve, part of the saphenous artery was also exposed to the light irradiation.

RESULTS

To determine whether the surgical and recording procedures would damage the saphenous nerve, identical surgical and recording procedures were performed on a control dog (no drug, no light, $n = 1$) and on contralateral control limbs (no light, $n = 5$) immediately and 1 week after concluding the PDT procedures on the treated limb. The configuration of the evoked CAPs and the conduction velocities elicited from the saphenous nerve at both the initial control surgery and at the 1 week follow-up were similar (pre-treatment conduction velocity of 50.61 ± 1.8 m/s vs. 54.33 ± 0.57 m/s post-treatment, pre- and post-treatment threshold values of 0.02 mA).

To determine how the light and its possible thermal effect would affect saphenous nerve conductivity, the contralateral control leg (no drug, $n = 2$) was exposed to escalating fluence rates (117, 150, or 300 mW on 1-cm linear diameter spot). Evoked CAPs were evoked and recorded during light irradiation approximately 2 min apart. The configurations and latencies of onset of the evoked CAP during light irradiation remained the same as the baseline. Tissue temperature increases of up to 5°C were observed at 300 mW. The conductivity velocity was increased slightly due to the temperature increase. When the light was turned off, the tissue temperature and nerve conductivity velocity returned to baseline levels. Light alone did not cause immediate CAP changes (data not shown). It is not expected that the small-scale temperature change in the irradiated area (1-cm diameter spot) would have a significant effect on the conduction velocity over the whole length of the saphenous nerve. Although the temperature data needs to be further validated, the presence of increased conduction velocity with increased nerve temperature has been well established.¹⁶

Treatment with 2 mg/kg of photosensitizer and 50 J/cm² ($n = 1$) produced little change. CAP conduction velocities remained the same before, during, and immediately after PDT, indicating no immediate PDT effect. Mild connective tissue proliferation around the treatment site was evident at 1 week post-PDT. Slight edema around the nerve was seen proximal to the irradiation site. The configuration and conduction velocities of the evoked CAPs both before PDT and at the 1-week follow-up were similar: 55.88 ± 1.26 m/s (33.9°C, during PDT) compared to 55.03 ± 0.14 m/s (34.9°C, 1 week post-PDT). The amount of current needed to reach threshold

stimulation changed from 0.1 mA measured immediately post-PDT to 0.15 mA 1 week post-PDT.

Treatment with 1 mg/kg photosensitizer and 100 J/cm² ($n=2$) produced no immediate change in nerve conduction velocity, but some changes in conduction velocity occurred 1 week post-PDT (Fig. 2). Mild edema along the incision line and nerve course was observed. In one dog, the saphenous artery had adhered to the adjacent saphenous nerve in the PDT-treated region. The configurations of the evoked CAPs both before PDT and at the 1-week follow-up were similar, but the conduction velocities were slightly decreased (7-18%). The leading CAP peak was of longer duration, possibly indicating slowing in conduction of some of the most rapidly conducting nerve fibers. The threshold stimulating current was approximately twice the current that was needed before PDT. Maximum response was reached at higher stimulation current. Therefore, the treated saphenous nerve still maintained its conductivity. The treated nerve section was able to conduct a signal, but higher threshold stimulation and amplification factors were required to elicit and record an evoked CAP. Nevertheless, such alteration of nerve function was localized to the treated area, whereas threshold values and velocities outside the irradiated site were comparable to pre-treatment values. These CAP profile changes at 1 week post-PDT indicate minimal and localized photodynamic effects on nerve conduction at this dose level.

Treatment with 1 mg/kg photosensitizer and 200 J/cm² ($n=3$) produced no immediate change in CAP conduction velocities but did produce marked changes at 1 week post-PDT. Vasculature surrounding the saphenous nerve appeared irritated. Edema along the incision line and nerve course was obvious. The treated nerve looked swollen, and individual fascicles were not as visually distinct as they were before PDT. Two out of three treated nerve sections were able to conduct a signal, but higher threshold stimulation (two- to fivefold current increase), higher current required to reach maximum amplitude (tenfold current increase) and higher amplification factors (greater than fivefold amplification factor) were needed to produce the evoked CAP and maximize CAP peaks. One treated nerve section produced no evoked potentials with the stimulating electrode proximal to the treatment site and the recording electrode placed either in the irradiation site or distal to the treatment site, indicating that PDT-induced damage was no longer localized. These CAP profile changes at 1 week post-PDT indicated significant photodynamic effect on nerve tissue and its conduction at this dose level.

Treatment with 2 mg/kg photosensitizer and 200 J/cm² ($n=2$) produced no immediate change but definite changes at 1 week post-PDT. In one dog, generalized cellulitis appeared around the nerve and surgical site 1 week after treatment. Another dog showed more severe injury, with a marked decrease in the diameter of the nerve in the treated area. The nerve looked edematous, with a serum pocket located proximally. No CAPs were produced within the PDT treated site during the 1 week follow-up; however evoked potentials were detected with both the stimulating and recording electrodes placed either proximal or distal to the original surgical site. In one dog, an evoked potential was produced with both the stimulating and recording electrodes placed proximal to the surgical site; however no CAP was detected with both electrodes located distally, indicating that PDT induced damage was no longer localized.

DISCUSSION

In this study, the effects of Tookad PDT on peripheral nerve tissue were evaluated using an *in situ* canine model. Evoked and recorded CAPs in the distal portion of the saphenous nerve were determined in animals receiving light control, drug control, and superficial PDT procedures. The PDT procedures were performed at various drug dose and light dose levels. Light dose levels of 100 and 200 J/cm² were delivered with a drug dose of 1 mg/kg, and 50

and 200 J/cm² were delivered with a drug dose of 2 mg/kg. This study demonstrated that Tookad PDT-induced conductivity changes were dependent on both drug dose and light dose.

Treatment of the saphenous nerve with light only, Tookad only, or a lower light dose, i.e., 50 J/cm² with a drug dose of 2 mg/kg, produced little nerve tissue damage or conductivity change. Treatment of the saphenous nerve with a light dose of 100 J/cm² and drug dose of 1 mg/kg produced mild edema and minor conductivity changes. At 1-week follow-up, the treated nerve was able to conduct a signal, but higher threshold stimulation and amplification factors were required to produce an evoked CAP. Alteration of nerve function was localized to the treated area, demonstrated by slower conduction velocities within the region, whereas threshold values and velocities outside the irradiated site were comparable to pre-treatment values. These CAP profile changes at 1 week post-PDT with 100 J/cm² indicate minimal and localized photodynamic effect on nerve conduction at this dose level despite evidence of increased vascular impairment. Mild surrounding tissue changes around the saphenous nerve did not appear to alter conduction properties.

The small variations in conduction velocity and threshold stimulation can be attributed to slight differences in recording conditions. Treatment of the saphenous nerve with a light dose of 200 J/cm² and drug dose of 1 mg/kg produced marked damage to the nerve and surrounding tissue along with conductivity changes. Edema is a characteristic of Tookad PDT-induced acute lesions, indicating severe tissue damage associated with higher drug/light doses. In two cases, increased stimulating current and amplification factors were required to produce an evoked CAP. These CAP profile changes at 1 week post-PDT indicate significant photodynamic effect on nerve tissue and its conduction at this dose level. One dog showed a total loss of conductivity, indicating that the nerve/surrounding tissue damage might be no longer localized to the treated area at this dose level. At an elevated drug dose of 2 mg/kg and 200 J/cm², PDT induced damages to the nerve and its surrounding tissues were visible, which were accompanied by a total loss of nerve conductivity within the irradiated site. Complete conduction loss distal to the irradiated site occurred in one dog, which might be due to vascular damage and interruption of blood flow outside the irradiated site.

Tookad is a second-generation photosensitizer that acts by damaging vasculature and altering blood supply to the irradiated area in current PDT protocols.^{2,3,15} The presence of proximal edema and reactive tissue in the treated area reflects its vascular acting nature. Since part of the saphenous artery is also exposed to light irradiation along with the saphenous nerve, it is reasonable to believe that the vasculature damage and surrounding tissue damage play significant roles in the total loss of conductivity at higher drug and light doses.

In conclusion, this canine model adequately demonstrates effects of Tookad PDT on peripheral nerves. Treatment with Tookad alone, light alone, or low-dose PDT (i.e., 50 J/cm²) produces very little or no change in nerve conduction properties. Direct irradiation of the saphenous nerve with 100-200 J/cm² light at 1-2 mg/kg drug dose can induce nerve damage and affect nerve conduction. Since these dose levels are likely used for prostate cancer patients, in order to preserve prostate nerve and minimize adverse effect on sexual and urinary functions in the process of total ablation of prostate cancer, possible side effects of interstitial PDT on pelvic plexus need to be further investigated.

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FIG. 1. Experiment setup. Black insulator was also used for blocking light to the tissues beneath the saphenous nerve.

FIG. 2.

CAP configuration changes before and after PDT. The dog was infused with 1 mg/kg of Tookad and irradiated with 100 J/cm² at 763 nm. Recording conditions for both traces include a stimulation rate of 4 pps, duration of 5 μ s, low-frequency filter of 10 Hz, high-frequency filter of 10 KHz, $\times$ 200,000 amplification, and 1500 data points.