MONITORING THE EFFICIENCY OF PHOTODYNAMIC THERAPY IN TISSUE

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ABSTRACT

Transcutaneous oxygen electrodes are used to non-invasively measure tissue oxygen tension during photodynamic therapy (PDT). Measurements are performed on VX-2 skin carcinomas in rabbit ears. The degree of tumor oxygen tension reduction is proportional to the applied light dose. In the absence of irradiation, oxygen tension returns to pre-irradiation levels until a "damage threshold" has been reached. For 50mW/cm^2 irradiations of Photofrin II (at 630 nm) and tetraphenylporphine tetrasulfonate (at 657 nm), the cumulative dose required to irreversibly deplete tumor transcutaneous oxygen was approximately 300 kJ/m^2 and 600 kJ/m^2, respectively.

1. INTRODUCTION

Photodynamic therapy (PDT) of neoplastic tissue is based on the administration of exogenous phototherapeutic agents, which are preferentially localized in that tissue and can be activated by radiation at suitably chosen wavelengths.[1]

It is generally agreed upon that oxygen plays a crucial role in PDT. Irradiation of the photosensitizer generates highly active oxygen intermediates, primarily singlet molecular oxygen, $^1O_2$ (at the expense of its precursor, ground-state molecular oxygen, O_2). This short-lived species is cytotoxic and can photooxidize essential cellular components.

Some photosensitizers act primarily on the tumor microvasculature and we will term the ensuing three-phase process "vascular PDT". Early-stage (phase I) vascular PDT is characterized by singlet oxygen-induced pathophysiological alterations of vascular cells. In phase II, these alterations lead to occlusion of blood vessels and hypoxia. In phase III, vascular collapse causes regional breakdown of blood supply and leads to tumor regression and necrosis.

There is also evidence that phototherapeutic drugs are localized in tumor cells. This may be due to specific structural properties of the photosensitizer (e.g., stereospecific functional groups, charge distribution, presence of metal ions) or physical-chemical properties (e.g. lipophilicity, aggregation) which will cause the drug to be taken up (or retained) in different biological compartments.[2] The ensuing "tumor-cell PDT" process leads directly to necrosis via sustained phase-I photooxidative damage to vital subcellular targets.

Irrespective of the exact PDT mechanism, whether tumor-cell or vascular, all phases of PDT are characterized by depletion of ambient oxygen in neoplastic tissue, either due to photooxidation or due to decreased O_2 supply through occluded blood vessels. Therefore, monitoring the oxygen tension in tissue during PDT is of great clinical importance, since it allows us to determine, in real time, the effectiveness of the therapy. Accordingly, we report here transcutaneous oxygen ($PtcO_2$) measurements for Photofrin II (PII) and tetraphenylporphine tetrasulfonate ($TPPS_4$) during PDT.

2. MATERIALS AND METHODS

The animal and PDT procedures were similar to those described previously.[3] Briefly, measurements were conducted on transplanted VX-2 skin carcinomas grown in the dorsal medial portion of New Zealand white rabbit ears. Phototherapy was initiated 24 h after 10 mg/kg body weight intravenous PII (Photomedica Inc., Raritan, N.J., lot PC2350) or $TPPS_4$ (Porphyrin Products, Logan UT) injections. Tumors (7-10 mm diameter) were irradiated with 90 mW of either 630 nm (PII cases) or 657

nm ($TPPS_4$ cases) laser light via a microlens-terminated optical fiber. Light was administered to the ventral side of the ear, opposite the electrode, in a 1.5 cm-diameter spot (50 mW/cm^2). "Laser on" times were either 60 s (30 kJ/m^2) or 100 s (50 kJ/m^2) for $TPPS_4$ and PII cases, respectively. "Laser off" intervals were twice the "on" times.

Transcutaneous oxygen electrodes (Novametrix Corp., Wallingford, CT) were stabilized, in all cases, for 20-60 minutes prior to irradiation. During this period the electrode heating unit raised the skin surface to 44 °C, effectively melting the stratum-corneum layer barrier to oxygen diffusion.[4] Control subjects received light without drug. Under these conditions, electrode response variations were negligible.

3. RESULTS AND DISCUSSION

In a recent study we demonstrated that transcutaneous oxygen electrodes can be used to monitor tissue oxygen disappearance during PDT.[3] With the drug Photofrin II (PII) it was shown that for the conditions of the experiment (tumor size/location and drug dose) a light dose of as little as 200 kJ/m^2 at 630 nm could cause irreversible tissue hypoxia. Figure 1 indicates that during cyclic irradiation (doses of 50 kJ/m^2, delivered in 100 s "laser-on" periods, each followed by a "laser-off" recovery time of 200 s), $PtcO_2$ is modulated by the appearance of laser light and exhibits an overall downward trend. Four or five "laser-on" periods appeared to be sufficient to elicit irreversible, phase-III hypoxic conditions

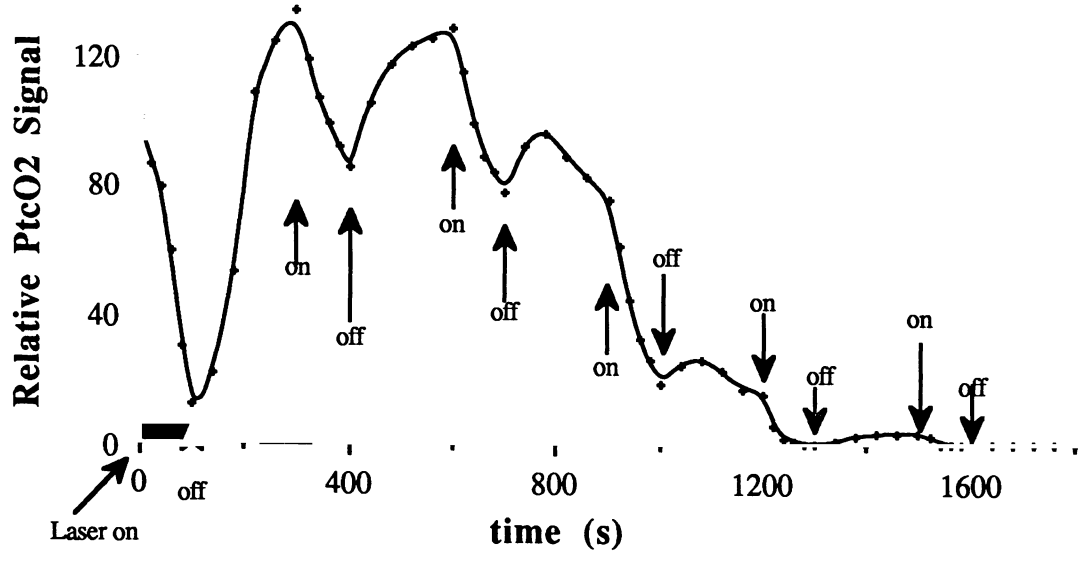

Figure 1. Transcutaneous electrode response (Relative % Oxygen pressure vs. time) for cyclic irradiation of rabbit ear tumor. Phototherapy conditions: 630 nm, 50 mW/cm^2; 50 kJ/m^2 each cycle. Photosensitizer: PII.

In contrast to the clinical drug of choice, PII, which is a partially purified multimeric and lipophilic aggregate, the synthetic, tumor-localizing drug tetraphenylporphine tetrasulfonate ($TPPS_4$) is well-defined, monomeric, anionic and hydrophilic.[5] $TPPS_4$ has been reported to be localized primarily inside tumor cells with negligible quantities retained in the endothelial cells.[6,7]

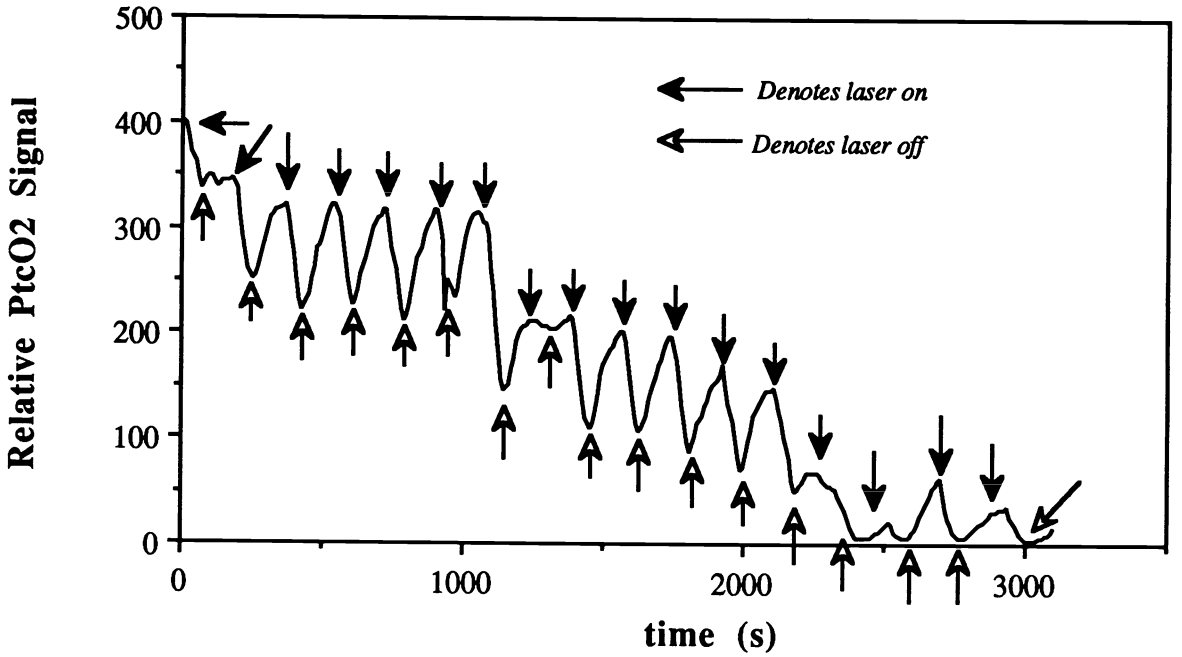

Figure 2. Transcutaneous electrode response (Relative % Oxygen pressure vs. time) for cyclic irradiation of rabbit ear tumor. Phototherapy conditions: 657 nm, 50 mW/cm^2; 30 kJ/m^2 each cycle. Photosensitizer: $TPPS_4$.

Figure 2 shows a typical response curve for $PtcO_2$ in $TPPS_4$-containing tumors obtained under intermittent irradiation: 60 s (30 kJ/m^2) "laser on" alternated by 120 s "laser off" periods. Unlike the case of PII-PDT, where as few as four cycles caused irreversible anoxic conditions, light-induced $PtcO_2$ modulations in $TPPS_4$ tumors seem to continue for many "laser-on-off" cycles.

Actual response differences between PII and $TPPS_4$ are illustrated, as a function of cumulative light dose, in Figure 3. Relative $PtcO_2$ levels were measured immediately prior to each irradiation. Differences in the mode of action of each drug are underscored by variations in the dose curves. Whereas PII signals drop sharply to near-zero levels within 300 kJ/m^2, $TPPS_4$ response is more gradual and tapers to zero levels after approximately 500-600 kJ/m^2.

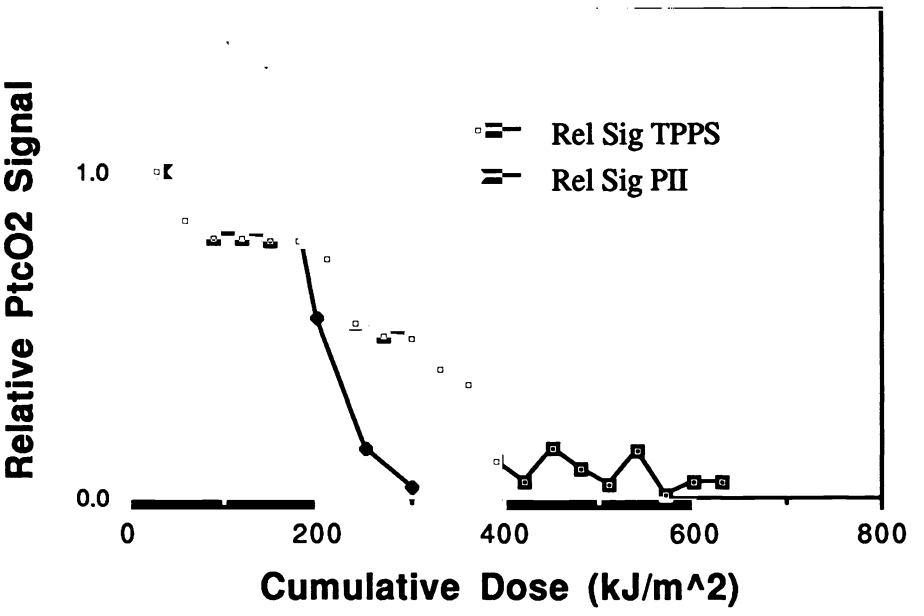

Figure 3. Relative $PtcO_2$ level (prior to each irradiation cycle) vs. cumulative light dose (kJ/m^2) for animals which received PII and $TPPS_4$.

From previous results we concluded that PII-PDT acts primarily on the tumor vasculature.[3] This is in accordance with independent reports of PII-induced microvascular damage observed *in vivo*[8,9,10] or histologically[11,12]. On the cellular level, these results indicate that PII is preferentially taken up by erythrocytes, platelets, endothelial cells and subendothelial membrane in and around the blood vessels, which then form the primary target for photodynamic attack.[12,13]

It is clear that the results for $TPPS_4$-PDT presented here are significantly different from those of PII-PDT. This difference may be due to varying contributions of "vascular" versus "tumor-cell" effects. For tumor-cell PDT, photogenerated singlet oxygen is consumed as long as blood flow persists. This shows up as a continuous depletion of tissue oxygen. The process is expected to continue as long as there remain (sub)cellular components which can serve as oxidizable targets for reaction with singlet oxygen. Under these conditions, $PtcO_2$ may not be a suitable criterion for predicting the minimum effective radiation dose, since oxygen depletion could be observed even after tumor cell death has occurred.

Alternatively, variations in $PtcO_2$ response between these compounds may simply be reflective of their relative "*in-vivo* efficiencies" for a given light and drug dose. In either case, measurements of the kind described here can be instrumental in predicting and understanding PDT efficacy and, perhaps mechanisms of action. In order to fully understand the latter, however, independent determinations of tissue damage, vascular changes, and drug uptake/distribution must be considered.

4. ACKNOWLEDGEMENTS

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