

## **PDT with ALA/PPIX is enhanced by prolonged light exposure putatively by targeting mitochondria.**

Steven L. Jacques 1  
Sergio Furuzawa 2  
Tom Rodriguez 3

1 Oregon Medical Laser Center, Portland, OR 97225

2 UNIVAP, Los Campos de San Jose, Brazil

3 Univ. Texas M. D. Anderson Cancer Center, Houston, TX 77030

### **ABSTRACT**

The synthesis of protoporphyrin IX (PPIX), a photosensitizer, occurs in the mitochondria of cells. Continuous light exposure activates the PPIX while it still resides in the mitochondria. The mitochondria are especially sensitive sites for lethal damage. In tissue culture experiments, cells were treated by PDT in two ways: (1) the cells were preloaded with PPIX by overnight incubation in medium containing ALA then exposed to light for various time periods, and (2) cells were not preloaded but rather were placed in medium containing ALA at the start of light exposure. Light exposure was 100 mW/cm<sup>2</sup>. After exposure the cells were plated and incubated for seven days to watch for colony formation. The results of experiment 1 showed that in preloaded cells, the large amount of PPIX that accumulates was easily photobleached in just 5 min which yielded a drop in cell survival to a level of 2%. Further light exposure up to an hour had no additional effect on cell survival. But when light exposure was continued past one hour, cell survival began to drop again. The results of experiment 2 showed that light exposure had no effect in the first hour, but thereafter continued exposure caused continued exponential drop in cell survival. In both experiments, cell survival dropped to 1/e its value for every 8 min of continued light exposure.

### **1. INTRODUCTION**

Although the use of ALA-induced PPIX as a photosensitizer is attractive, there have been complaints about the amount of PPIX that accumulates in tissues and the rapid photobleaching that occurs. The combination of low PPIX and rapid photobleaching yields a low net PDT dose which may fail to achieve sufficient cell killing during a short light exposure that photobleaches all accumulated PPIX.

This paper discusses the use of prolonged light delivery to utilize PPIX as it is made within the mitochondria (Figure 1). The mitochondria are especially sensitive sites for lethal damage. By activating PPIX while still in the mitochondria, the effectiveness of PPIX is maximized. And continued PPIX synthesis presents a fresh supply of PPIX for activation throughout the exposure.

Oxygenation is also an issue in PDT. Too rapid a PDT treatment (either too much photosensitizer or too much light power density) can drive the complete conversion of molecular oxygen to singlet oxygen and deplete the molecular oxygen concentration in the tissue between capillaries and outpace the diffusion of molecular oxygen from the capillaries into the tissue. Prolonged light exposure is by definition a slow process which does not outpace oxygen delivery to tissue.

## **Targeting mitochondria with PDT using ALA/PPIX and prolonged light exposure**

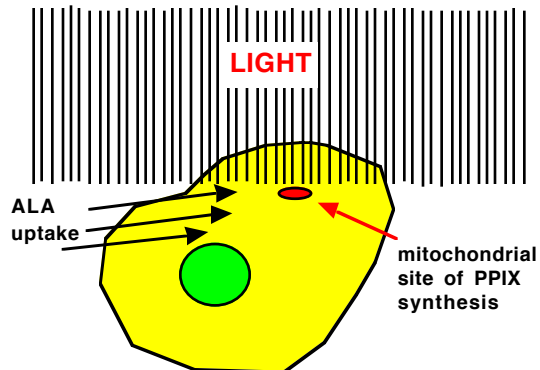


Figure 1: Continuous light exposure activates the PPIX as it is synthesized with the mitochondria.

## **2. METHODS**

Cancer cells in tissue culture (MTF7 mammary cell ca., Stephen Tomosovic, MD Anderson Cancer Center) were treated in two ways.

Experiment 1: Cells were incubated in 200  $\mu\text{g/ml}$  ALA, the precursor molecule for PPIX synthesis, for 24 hr. Then cells were loosened by mild Trypsin digestion, washed, and placed in a small volume of medium. Media during light exposure were tested with and without ALA and surprisingly no significant difference was seen in the results. Cells were stirred while exposed to 100  $\text{mW/cm}^2$  of 630 nm light delivered via optical fiber and lens system from an argon/dye laser. At various times of light exposure, aliquots of cells were removed and plated for a 7-day incubation. After a 7-day incubation, cell colony formation was scored.

Experiment 2: The same as experiment 1, except the cells were not preloaded with ALA. Rather, they were first exposed to ALA when placed in the stirred medium at the commencement of light exposure.

### **METHOD**

Expt A: ALA added here

- **Grow MTF7 mammary carcinoma cells in culture for 24 hr.**
- **Loosen growing cells from plate with mild trypsin digestion.**
- **Resuspend in fresh medium.**

Expt B: ALA added here

- **Irradiate aliquots of cells by laser for increasing times (630 nm, 100  $\text{mW/cm}^2$ )**
- **Transfer cells to culture dish and incubate for 7 days**
- **Count colony formation**

### 3. RESULTS

#### 3.1 Experiment 1

The results of the experiments are shown in Fig. 2.

The first 5 min of light exposure photobleached the pre-accumulated PPIX and caused rapid killing of about 98% of the cells. The initial solid line in the figure is

$$\text{Survival} = 0.02 + 0.98\exp(-tE/(7 \text{ J/cm}^2))$$

where E is 0.1-W/cm<sup>2</sup> irradiance at 630 nm and t is time in [s]. Then up to 35 min of light exposure, no further drop in cell survival was seen. All the PPIX had been photobleached. However, beyond 35 min, newly synthesized PPIX apparently began to be activated by the light and cause loss of cell survival:

$$\text{Survival} = 0.02 + 0.98\exp(-t/(8 \text{ min}))$$

Our working hypothesis is that this 8 min time constant characterizes the rate of new PPIX synthesis.

In experiment 1, the cells were placed in media without ALA for the light exposure. One experiment included ALA in the media during light exposure. There was little difference in the survival with or without ALA in the medium, suggesting that sufficient ALA was retained to maintain PPIX synthesis. This observation needs further attention.

In experiment 2, there was no effect of light exposure during the first 60 min when presumably the enhanced PPIX synthesis due to the ALA in the medium was first beginning. At 60 min, the PPIX synthesis apparently had achieved sufficient enhancement that cell killing began. The drop in cell survival with prolonged light exposure beyond 60 min was a drop to 1/e its value for each ~8 min of exposure, just as in experiment 1. In our experience with MTF7 cells, light alone has little effect on cell survival.

#### Cell survival following prolonged exposure PDT:

**expt 1 = cells preincubated 24 hr in ALA before light**

**expt 2 = cells given ALA at start of light exposure**

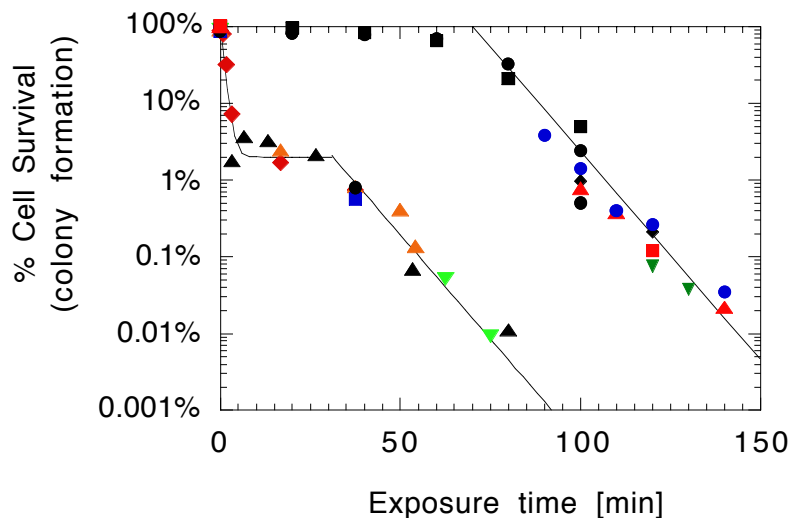


Figure 2: Cell survival in experiment 1 after PDT using ALA/PPIX. Cells had been preloaded with PPIX by 24 hr preincubation in ALA. Each symbol denotes a separate experiment on different-day.

#### 4. DISCUSSION

Prolonged light exposure avoids the problems of non-uniform light delivery. The dosimetry is limited by the available PPIX, not by the available photons. As long as the central region between two light sources is sufficiently irradiated to photobleach all synthesized PPIX, then the PDT treatment will be uniform. Excess light without PPIX to be activated does not affect cell survival. (Figure 3)

**Dosimetry depends on metabolic rate of PPIX synthesis, not on tissue optics.**

Avoids problem of nonuniform light delivery:

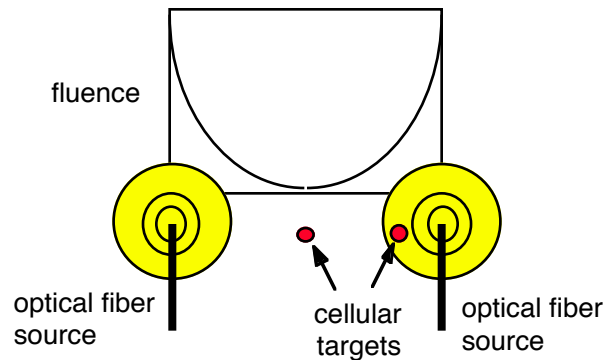


Figure 3: Prolonged light exposure avoids the problems of non-uniform light delivery.

Prolonged light exposure avoids the problems of depleting molecular oxygen. When a PDT treatment is driven too fast (either too much photosensitizer or too much light), then molecular oxygen is converted to singlet oxygen and the slower process of oxygen diffusion from capillaries cannot keep pace with the PDT. The result is PDT is less effective due to lack of oxygen. A prolonged light exposure that depends on the slow synthesis of PPIX sensitizer avoids the depletion of oxygen. (Figure 4)

Prolonged exposure allows time for diffusion of oxygen to the site, replenishing oxygen used by the PDT.

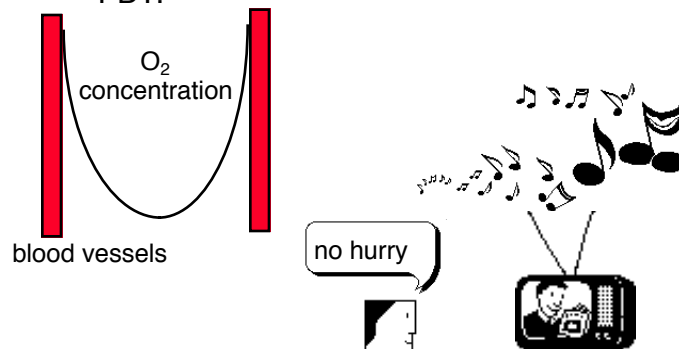


Figure 4: Prolonged light exposure avoids the problems of depleting oxygen.

## 5. CONCLUSIONS

- Prolonged light exposure allows PPIX to be activated when first synthesized within the mitochondria which is a sensitive site for PDT effect.
- The prolonged light exposure allows continued synthesized PPIX and continued PDT effect after the initial PPIX has been photobleached. The cell killing continues to drop with continued light exposure.

### ALA/PPIX

+

### prolonged light exposure

**achieves much greater killing efficiency than acute treatment.**

- **activate PPIX in sensitive sites (mitochondria)**
  - **uniform light delivery is not required**
  - **local oxygen is maintained by O<sub>2</sub> diffusion**
  - **ALA administration can be fractionated**
- 
- **Loss of cell survival decreases as**  
**survival  $\approx \exp(-t/8 \text{ min})$**

This work has begun to be applied to treatment of subcutaneous MTF7 tumors in a rat model. Three-hour light exposures with 200 mg/kg ALA injections at 0 and 1 hr have achieved very strong tumoricidal effects, stronger than has been our previous experience with this rat model using PPIX or other sensitizers with acute light exposure (10-20 min).

THIS WORK WAS SPONSORED BY THE NIH (HL45045) AND THE DEPT. OF ENERGY (DE-FG03-95ER61971).