Photodynamic Therapy of High-Grade Cervical Intraepithelial Neoplasia With 5-Aminolevulinic Acid

Kristin A. Keefe, MD,2 Yona Tadir, MD,1 Bruce Tromberg, PhD,1 Michael Berns, PhD,1 Kathryn Osann, PhD,1,2 Rasha Hashad, MD,1 and Bradley J. Monk, MD2*

1The Beckman Laser Institute, University of California, Irvine, California
2Division of Gynecologic Oncology, Department of Obstetrics and Gynecology at the Chao Family Comprehensive Cancer Center, Orange, California

INTRODUCTION

Although cervical cancer is one of the leading causes of cancer in women worldwide accounting for more than 400,000 deaths per year [1,2], the recognition and treatment of cervical intraepithelial neoplasia (CIN) as a precursor lesion through Pap testing has all but eradicated invasive cervical cancer in developed countries [2]. Once identified through colposcopic directed biopsies during the evaluation of an abnormal Pap, CIN is treated with locally ablative techniques such as cryotherapy and laser ablation, or excision. Unfortunately, such techniques are ablative to both non-neoplastic tissue as well as the pre-cancerous CIN. However, although the optimal method of ablating/excision of CIN is not known, all of these methods as well as others have little morbidity and reasonable efficacy [3].

In order to develop a novel locally ablative modality, which would be highly specific to dysplastic cervical epithelium preserving normal cervical tissue and architecture thereby potentially decreasing acute and chronic adverse effects, we have investigated the use of photodynamic therapy (PDT) as a treatment for CIN. PDT is a technique in which visible light is used in combination with a photosensitizing agent to achieve cytotoxicity [5,6]. The most commonly used photosensitizers are hematoporphyrins such as dihematoporphyrin ether (DHE), the active fraction of hematoporphyrin, which in clinical practice is usually injected intravenously (IV). DHE is concentrated in various tissues after injection predominantly in the liver followed by the spleen, kidney, dysplastic and frankly invasive tumor cells, skin, muscle, brain, and lungs [7]. Due to this differential localization of the photosensitizing agent in neoplastic versus normal epithelial cells, PDT can be used to selectively ablate preinvasive and invasive neoplasms. PDT requires activation of the photosensitizing agent by laser light at a specific wavelength and the photosensitizer is activated to a toxic photodynamic state by the laser. The photosensitizer induces a destructive reaction in the cells, usually by a singlet oxygen mechanism, resulting in cytotoxicity to the target tissue.

Study Designs/Materials and Methods: Forty women, who were at least 18 years old with persistent biopsy-proven CIN 2 and CIN 3 within the previous 3 months of enrollment, underwent PDT in a phase I and II design. Five escalating radiant energies (increments of 25 J/cm², beginning at 50–150 J/cm²) using a Coherent Dye Model 920 argon pumped dye laser providing light at 630 nm (maximum output 0.8 W) were used to perform PDT with a fixed dose of ALA (200 mg/ml). ALA was placed in a cervical cap fitted to the cervix. After 90 minutes, the cap was removed and the ectocervix was illuminated for 5–16 minutes, depending on the irradiance. Success was defined as the absence of CIN on Pap smear or colposcopic examination at 12-months. Patients were monitored for toxicity.

Results: Thirty-two women (80%) completed the study with 1 year of follow-up. Sixty percent had CIN 3 and 40% CIN 2. Success rates at 4, 8, and 12 months were 51, 46, and 31%, respectively, and were not light-dose dependent. Three patients progressed from CIN 2 to CIN 3. Toxicity was tolerable and only consisted of spotting, vaginal discharge, mild cramping, and vaginal warmth. There was no apparent dose relationship to toxicity.


Key words: gynecology; dysplasia; laser; photodynamic therapy; 5-aminolevulinic acid

Grant sponsor: National Cancer Institute (to M.B. and B.J.M.);
Grant numbers: CA-32248, 1K23CA87558.
*Correspondence to: Bradley J. Monk, MD, UCI Medical Center, 101 The City Drive, Bldg. 23, Rm. 107, Rt. 81, Orange, CA 92868; E-mail: bjmonk@uci.edu
Accepted 26 July 2002
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.10111
agent by a light; usually a red light at a wavelength of 630 nm delivered by an argon pumped dye laser because of its greater tissue penetration. The mechanism of action of PDT involves the formation of singlet oxygen, which oxidizes biologic molecules causing irreversible subcellular damage with destruction of tumor vasculature, causing anoxia and necrosis. Because of its selectivity and tumoricidal effect, we expanded on the traditional methods of systemic DHE administration and PDT by developing techniques to apply DHE and its second-generation derivatives directly to the cervix.

In 1997, we reported the first phase I investigation of locally applied DHE in the PDT management of CIN [8]. Through the application of 2 ml of a 1% solution of DHE in a 4% Azone and isopropyl alcohol vehicle applied to the cervix for 24 hours, the common systemic toxicity of cutaneous photosensitivity associated with intravenous DHE was avoided. Phase II studies using a constant irradiance (150 mW/cm²) to avoid thermal injury at a PDT radiant exposure of 140 J/cm² were recommended. Although this phase I study was not designed to evaluate efficacy, the 68% response rate among all PDT energies studied was encouraging. However, women with CIN I were included in this study compounding the interpretation of the efficacy data since a high rate of spontaneous regression is well documented among women with low-grade dysplasia [9].

5-aminolevulinic acid (ALA) is a second-generation photosensitizer and an attractive photosensitizing agent for PDT. Its photoactive derivative, protoporphyrin IX, is metabolized within 1–2 days, eliminating prolonged skin photosensitivity even when administered IV. The objective of this study was to evaluate the safety and efficacy of ALA as a topically applied photosensitizer for PDT of persistent biopsy-proven CIN 2 and 3 using a prospective phase I/II study design.

MATERIALS AND METHODS

Women 18 years of age or older not planning on becoming pregnant within 1 year and with biopsy-proven CIN 2 or 3 within 3 months of enrollment were eligible for this study. All lesions were colposcopically visible without suspicion for invasion or extension into the endocervical canal. A negative endocervical curettage and pregnancy test were required and lesions were confirmed to be persistent by colposcopy the day of PDT. Those with a poor performance status (Southwest Oncology Group Performance status ≥ 2), a history of DES exposure, and a history of an invasive malignancy within the last 5 years or previous treatment of CIN in the last 3 months were excluded. Women were required to have no history of cutaneous photosensitization, porphyria, or hypersensitivity to porphyrins or Hyskon [8]. Eligible patients underwent a screening interview, and signed an Institutional Review Board approved consent prior to treatment.

After confirmation of the lesion, PDT was performed using escalating radiant energies (50, 75, 100, 125, 150 J/cm²). Six women were treated at each light dose in a phase I dose escalating fashion. Six women were treated at each level to assess rare toxicities generally not seen with phase I trials studying fewer patients. In addition, ten more women (16 total) were treated at the maximum tolerated dose to provide limited phase II efficacy data at that level. Discontinued enrollment into any light dose group was planned if unacceptable toxicity including ulceration, intractable pain, cervical stenosis, and treatment-related hospitalization were encountered. A fixed drug dose of ALA (200 mg/ml) was used. Crystallized ALA hydrochloride (DUSA Pharmaceuticals, Inc., Denville, NJ) was diluted to 200 mg/ml in Hyskon (Pharmacia, Inc., Piscataway, NJ) prior to application. The solution was titrated to pH 6 with 10 N sodium hydroxide. Hyskon is a viscous, hydrophilic, branched polysaccharide, and has been used in several animal and human studies for the topical administration of ALA in the endometrium and the uterine cervix [15,16,17]. PDT was performed with the patient in the dorsal lithotomy position and without anesthesia. Light at 630 nm provided by the laser system described elsewhere [8] was coupled into a 400 µm silica fiber optic terminating in a microlens which focused the laser radiation onto a 10–30 mm diameter circular field of uniform irradiance perpendicular to the tissue. A custom made vaginal speculum was manufactured [8], which allowed stabilization of the optical fiber and focusing of the light spot size by changing the distance of the fiber from the treatment area. The entire transformation zone was treated in a single field including a margin of 3–5 mm of normal ectocervix. Using a constant irradiance (150 mW/cm²) in order to avoid thermal injury, the radiant exposure was increased every six patients in a phase I fashion (50, 75, 100, 150 J/cm²).

Radiant exposure was escalated by increasing the exposure time at a given power output as follows: 50 J/cm², 5’33”; 75 J/cm², 8’20”; 100 J/cm², 11’07”; 125 J/cm², 13’53”; 150 J/cm², 16’40”.

Patients were monitored for systemic as well as local toxicity 1 and 4 weeks after therapy. Response was assessed colposcopically and cytologically 4, 8, and 12 months after PDT. Colposcopically directed biopsies of all abnormal areas were performed. Patients were removed from study and treated by other means (Laser or LLETZ) if biopsy proven CIN II or III was documented 4, 8, or 12 months after PDT. Progression was defined as CIN 2 to 3 or CIN 3 to cancer or the development of new lesions. Those who refused or were unable to continue in the study were removed.

The primary endpoint of the study was toxicity and persistent resolution of CIN (no evidence of CIN on Pap smear, endocervical curettage, or cervical biopsy after 12 months). Chi square analysis was used to compare proportions.

RESULTS

Forty women with a mean age of 27 years, including 65% Caucasian, 13% Asian, and 22% Hispanic were enrolled. No toxicity prevented progression through the study light-doses. Referring biopsies showed that 60% had CIN 3 and 40% had CIN 2 (Table 1). Thirty-two women (80%) completed the study with 1 year of follow-up. Four (10%)
withdrew for personal reasons not related to toxicity, and four (10%) were lost to follow-up (two at 4 months and three at 12 months). Overall success rates at 4, 8, and 12 months were 51, 46, and 31%, respectively, and were not light-dose dependent at 12 months (50, 20, 0, 50, and 29% at 50, 75, 100, 125, 150 J/cm², respectively). These results are indicated in Figure 1 and Table 2. Four patients progressed from CIN 2 to CIN 3. Minimal toxicity (grade 1) was reported and included spotting (n = 3), discharge and mild cramping after treatment. During therapy, only vaginal warmth (n = 4) was experienced. There was no apparent dose relationship to toxicity: below 125 J/cm², 5/18 discharge and 5/18 cramping; 125 J/cm², 5/6 discharge and 3/6 cramping; 150 J/cm², discharge 15/16 and 4/16 cramping.

**DISCUSSION**

PDT, due to its tumor selectivity, represents an alternative approach to diagnose and treat CIN without altering normal adjacent tissue. The PDT management of CIN has the distinct advantage of preserving normal non-neoplastic cervical tissue being directly cytotoxic to the dysplastic epithelium and does not require local anesthesia. Indeed, this approach has been both effective and well tolerated in other intraepithelial lesions such as Barrett’s esophagus [10] as well as carcinoma in situ of the bladder [11] and vulvar intraepithelial neoplasia [12,13]. PDT is a technique whereby light is used to achieve a toxic effect in photosensitized cells. When excited by red light in the presence of oxygen, photosensitizers such as ALA or DHE, hematoporphyrin derivatives, undergo a series of reactions producing a selective cytotoxic effect in areas where the photochemical reaction occurs. Energy from the excited triplet state of the photosensitizer, induced by the absorption of light of the appropriate energy and wavelength, is directly transferred to molecular oxygen producing singlet oxygen. Although it is very unstable and short lived, singlet oxygen is extremely reactive and capable of oxidizing biologic molecules causing irreversible damage to subcellular organelles such as the cell membrane, mitochondria and lysosomes [5,6].

Our group as well as others have shown that the topical administration of ALA to the cervix is safe [14] and results in its selective accumulation in HPV infected CIN after topical administration [15,18,19]. Selective uptake of ALA by CIN has been demonstrated by both quantitative fluorescence microscopy [15,19] and real-time image analysis [18]. Human papillomavirus (HPV) DNA positive lesions have shown the highest ALA induced fluorescence resulting in a ratio as high as 11:1 when normal and HPV infected tissues are compared [18].

<table>
<thead>
<tr>
<th>Radiant exposure (J/cm²)</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>125</th>
<th>150</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 3</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>CIN 2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>16</td>
<td>40</td>
</tr>
</tbody>
</table>

**TABLE 2. Twelve Month Outcome by Lesion Grade**

<table>
<thead>
<tr>
<th></th>
<th>Success</th>
<th>Failure</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 3</td>
<td>6</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>CIN 2</td>
<td>4</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>22</td>
<td>32</td>
</tr>
</tbody>
</table>

*P* = 0.93.

![Fig. 1. Efficacy at 4, 8, and 12 months according to radiant exposure.](image-url)
The systemic administration of a photosensitizer and PDT seems to be remarkably effective in treating CIN. Although well controlled studies are lacking, Muroya et al. from Japan reported an incredible 96% complete response rate. However, the utility of this approach is limited by skin photosensitivity [20]. Unfortunately, the effectiveness of PDT to treat CIN using topical photosensitizers has been much less encouraging. In 1997, we reported the results of a phase I trial using DHE. However, because newer more potent photosensitizers became available, further studies have focused on ALA. To date, only one study has shown topical ALA to be effective in the treatment of CIN. Wierrani et al. in Vienna treated 20 CIN I and II patients with topically applied 12% w/v ALA (with a cervical cap 8 hours prior to illumination). A thermal light source (150 W halogen lamp) emitting a broadband red light (fluence: 100 J/cm², fluence rate: 90 mW/cm²) was used for superficial illumination of the ectocervix. In addition, an Nd:YAG pumped dye laser (652 nm) was used to illuminate the cervical canal (total energy: 50 J/cm ², fluence rate: 300 mW/cm²). Preliminary results of their trial with a follow-up of 1, 3, 6, and 9 months post therapy showed cytological improvement in the grading of the Pap smears in 19 patients and the eradication of cervical HPV in 80% [21]. Importantly, the interpretation of this study is limited by the inclusion of low-grade lesions which have a high spontaneous regression rate, the small numbers of women treated, the short follow-up and the lack of a control arm.

Another smaller study of topical ALA suggested that it was not an effective therapy for high-grade CIN. In a preliminary report of seven patients, Hillemanns et al. in Germany treated seven women using fixed 5-ALA doses and application protocols derived from previous in vitro and in vivo results. Three to five hours prior to PDT, 10 ml of a 20% solution of 5-ALA was topically applied using a cervical cap. PDT was performed with irradiation of 100 J/cm² at an irradiance of 100–150 mW/cm² with an argon-ion-pumped dye laser at 635 nm. For the endocervix, a specifically designed cylindrical applicator was used. Ten treatment cycles of PDT using 5-ALA were performed in seven patients with high-grade CIN. Non-thermal laser treatment with 100–150 mW/cm² was well tolerated. Local toxicity was minor as several patients reported burning sensations and vaginal discharge, but no necrosis, sloughing or scarring occurred. After 3 months, a significant reduction in the size of the ectocervical CIN lesions was noted in only three patients, who underwent a second PDT cycle. However, no significant improvement in CIN lesions was noted since cold knife conization revealed persistent CIN in all seven cases [22].

Because of the conflicting results of these two preliminary studies of topical ALA and because of our encouraging phase I studies [8,15], we decided to perform a systematic phase I and II study to evaluate the safety and efficacy of ALA as a topically applied photosensitizer in the PDT of CIN 2 and 3 lesions. Care was taken to document persistent biopsy proven high-grade dysplasia and to conduct careful long-term follow up. Interestingly, success rates at 4, 8, and 12 months were disappointing and not light-dose dependent (50, 20, 0, 50, and 29% at 12 months and at 50, 75, 100, 125, 150 J/cm², respectively). Four patients progressed from CIN 2 to CIN 3. Importantly, minimal toxicity was reported similar to previous reports [21,22].

Five explanations for the negative results of the current study can be proposed. First, PDT may not be effective in treating biopsy proven CIN 2 and 3. The positive results of systemically administered photosensitzers in the treatment of CIN and other positive studies in other diseases including those using topically applied drugs contradicts this and only randomized trials using intravenous photochemicals in the treatment of CIN will answer this most important question. Next, the ALA concentration, time of ALA exposure, and/or light dose fraction may not have been optimal for effective PDT in the present study and thus might account for the lack of effectiveness.

Increasing concentrations of ALA can facilitate passive diffusion of ALA into the cervix. In the current study as well as Hillemanns’ negative study [22], a concentration of 20% (200 mg/ml) was used while Wierrani’s positive study used 12% [21]. Uptake studies have used concentrations as low as 1 [18] and 3% [19] with effective selective accumulation being demonstrated by fluorescence microscopy. A concentration of 0.05% has been shown to be too dilute [18]. Perhaps, increased doses are not necessary and future studies should probably use more dilute rather than more concentrated solutions.

Passive diffusion of ALA into the cervix not only depends on concentration but also time. Although Wierrani’s study [21] used 8 hours of ALA exposure, all other studies including the uptake studies [15,18,19] previously cited and Hillemanns’ study [18] as well as the current study have used exposure times of ~1.5 to 3 hours. This variable seems to be the least important in predicting efficacy.

The fourth and most complicated and perhaps the most important factor in PDT is the time–dose fractionation of light. Fractionation of light and prolongation of treatment time may allow increased blood flow to the area of interest thereby improving tissue oxygenation and PDT efficacy. On the other hand, it may decrease photosensitizer concentration thereby decreasing PDT efficacy. This delicate balance of time–dose fractionation has been studied in the normal rat colon. Experiments have examined the area of necrosis around a single light delivery fiber 3 days after PDT with a range of light-dose fractionation regimes. All animals were given 200 mg/kg ALA IV 2 hours prior to light delivery (100 mW at 635 nm) and each interruption in illumination was for 150 seconds. The area of PDT necrosis (total radiant energy 25 J) could be increased by a factor of three with a single interval after 5 J, compared with continuous illumination. Alternatively, with this single break, the total light dose could be reduced by 60% to achieve the same area of necrosis as with continuous illumination. This simple modification to PDT with ALA could markedly reduce current treatment times as well as increasing clinical efficacy [23]. Another improvement in the PDT treatment of CIN might be the addition of an Excimer Dye Laser (EDL), a type of low pulse laser, which has a considerably higher degree of tissue penetration because of its higher
PHOTODYNAMIC THERAPY OF HIGH-GRADE CIN 293

PHOTODYNAMIC THERAPY OF HIGH-GRADE CIN

PHOTODYNAMIC THERAPY OF HIGH-GRADE CIN

REFERENCES


CONCLUSIONS

Hence, multiple treatments may be necessary when topical PDT is used. Finally, photobleaching may be responsible for the negative results reported in the current study (24,25). Photobleaching results form the inactivation of the photochemical due to exposure to light, which is too intense (high irradiance). Unfortunately, a lower irradiant source would result in fewer photons per unit time available to activate the photochemical at a given depth. Thus, just enough but not too much light is critical to effective PDT. Studies addressing photobleaching of topically applied photochemicals to the cervix are lacking and indicated. In addition, future studies investigating cervical PDT should require random assignment to a control arm to determine the true efficacy of this new therapy. Moreover, lower doses of ALA than the current study, multiple treatments, endocervical illumination and alternative time dose fractionation should be incorporated into upcoming trials.

ACKNOWLEDGMENTS

Supported by Public Health Service grants from the National Cancer Institute to MB (CA-32248) and BJM (1K23CA87558).

PHOTOBLEACHING RESULTS FORM THE INACTIVATION OF THE PHOTOCHEMICAL DUE TO EXPOSURE TO LIGHT, WHICH IS TOO INTENSE (HIGH IRRADIANCE). UNFORTUNATELY, A LOWER IRRADIANT SOURCE WOULD RESULT IN FEWER PHOTONS PER UNIT TIME AVAILABLE TO ACTIVATE THE PHOTOCHEMICAL AT A GIVEN DEPTH. THUS, JUST ENOUGH BUT NOT TOO MUCH LIGHT IS CRITICAL TO EFFECTIVE PDT. STUDIES ADDRESSING PHOTOBLEACHING OF TOPICALLY APPLIED PHOTOCHEMICALS TO THE CERVIX ARE LACKING AND INDICATED. IN ADDITION, FUTURE STUDIES INVESTIGATING CERVICAL PDT SHOULD REQUIRE RANDOM ASSIGNMENT TO A CONTROL ARM TO DETERMINE THE TRUE EFFICACY OF THIS NEW THERAPY. HOWEVER, LOWER DOSES OF ALA THAN THE CURRENT STUDY, MULTIPLE TREATMENTS, ENDOCERVICAL ILLUMINATION AND ALTERNATIVE TIME DOSE FRACTIONATION SHOULD BE INCORPORATED INTO UPCOMING TRIALS.