Photomedicine in Gynecology

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Training Course for Advanced Oncologic Laparoscopy
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Photodynamic Principle

- Use of a photo-enhancing or photo-sensitizing chemical to aid in the diagnosis or treatment of a target cell
Clinical Studies in Gynecology

- **Condyloma** Fehr et al, Am J Obst Gyn 1998
- **Cervical and vulvar dysplasia** Hillemanns et al, Cancer 2000
PHOTORADIATION THERAPY OF CANCER
(Laser-Hematoporphyrin Derivative)

1. Cancer
2. 48 - 72 hours
3. Drug selectively retained by cancer cells
4. Inject HPD (drug) in vein

- Argon Laser (514 nm, 488 nm, Blue-Green Light)
- Dye Laser

Fiber optic bundle

- 625-635 nm (Red Light)

- Sens + hv → 1 sens*
- 1 sens* → 3 sens*
- 3 sens* + O₂ → O₂ + sens
- O₂ + substrate → oxidation
- sens = HPD

"O₂ Kills Cells"
PHOTODETECTION
Historical


1993  First approval (by the canadian health agency) of PDT with Photofrin® for the prophylactic treatment of bladder cancer.
Absorption of light

Radiative mechanisms

(1) Fluorescence
(2) Phosphorescence

Net effect

Light \rightarrow Light
\textit{hv} \rightarrow \textit{hv}'

Radiationless mechanisms

Chemical
(1) Singlet
(2) Triplet

Net effect

Light \rightarrow Chemistry
\textit{hv} \rightarrow \Delta G

Radiative mechanisms

Net effect

Light \rightarrow Heat
\textit{hv} \rightarrow Q
Photophysical Processes

Fluorescence detection

Photodynamic Therapy

Absorption

Spectroscopy

Photon energy levels:

$S_0 \rightarrow S_1 \rightarrow S_2$

$\tau = 1\text{ns}$

$\Delta d = 45\text{nm}$

$\tau = 10\mu\text{s}$

$\tau = 250\text{ns}$

$\text{Fluorescence}$

$\text{Phosphorescence}$

$\text{Singlet Oxygen production}$

Collision energy transfer

Energy levels:

$400\text{nm}$

$630\text{nm}$

$700\text{nm}$

$630\text{nm}$

$33\text{nm}$

$1^* \rightarrow 3\text{O}_2$
Photosensitizers

• Porphyrins
  – Photofrin (PF)
  – "Aminolevulinic acid (ALA)"
  – Protoporphyrin IX (PpIX)

• Chlorins
  – m-Tetrahydroxyphenyl chlorin (mTHPC)
  – Benzoporphyrin derivative mono-acid (BPD)
  – Tin ethyl etiopurpurin (SnET2)

• Phtalocyanines
# PDT with second generation PS

<table>
<thead>
<tr>
<th>PS</th>
<th>Dose (mg/kg)</th>
<th>D / L (hours)</th>
<th>WL (nm)</th>
<th>Light dose (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTHPC</td>
<td>0.075 - 0.15</td>
<td>96</td>
<td>652 (red)</td>
<td>5 - 20</td>
</tr>
<tr>
<td>ALA-PpIX</td>
<td>40 - 60</td>
<td>4 - 6</td>
<td>514 (green)</td>
<td>75 - 120</td>
</tr>
<tr>
<td>Topical 20%</td>
<td></td>
<td></td>
<td>635 and 405</td>
<td>10 - 200</td>
</tr>
<tr>
<td>BPD-MA</td>
<td>0.3</td>
<td>0.4 - 2</td>
<td>690 (red)</td>
<td>50 - 150</td>
</tr>
<tr>
<td>NPe6</td>
<td>0.5 - 1</td>
<td>4 - 8</td>
<td>(red and blue)</td>
<td></td>
</tr>
<tr>
<td>Lu-Tex</td>
<td>0.6 - 7</td>
<td>3</td>
<td>664 (red)</td>
<td>50 - 100</td>
</tr>
<tr>
<td>SnET2</td>
<td>1.2</td>
<td>24</td>
<td>732 (red)</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>660 (red)</td>
<td>200</td>
</tr>
</tbody>
</table>
Photofrin Approval

- Superficial bladder cancer (Canada 1993)
- Early and late oesophageal and lung ca (Netherlands 1994)
- Advanced oesophageal ca (USA 1995)
- Early ca of stomach, oesophagus, lung, cervix and cervical dysplasia (Japan 1994)
Haem Biosynthesis

5-ALA synthase

Uptake of exogenous 5-ALA

PBG Deaminase

Cytoplasm

Lower rate in tumour cells

Higher rate in tumour cells

Coproporphyrinogen III

Uroporphyrinogen III

Protoporphyrinogen IX

Protoporphyrin IX

Fe^{2+}

Feedback control

Ferrochelatase

Mitochondria

Glycine + succinyl CoA

5-ALA

haem
Absorption (dark line) and fluorescence (light line) spectrum of PpIX solved in DMSO. Values of absorption and fluorescence do not correspond to each other.
Penetration depth of light in tissue in relation to the wavelength
Absorption of water, melanin (broken line) and oxyhemoglobin (HbO2) (dotted line)
Figure 1

Common sites of ovarian cancer metastases.

Ovarian cancer spreads fast to the whole abdominal cavity by exfoliation.
5-Year cumulative lethality of gynecologic malignancies in Geneva
Epithelial Ovarian Cancer

- Fourth most frequent cause of “cancer-related” death
- 65% diagnosed with stage III-IV disease
- Initial response: 80% platinum sensitive
- 5 year survival rate: 15-20%
- Second look laparotomy
  - Historically: no effect on survival
    - 1/3 macroscopic
    - 1/3 microscopic
    - 1/3 negative
  - 50% of patients with a negative second look laparotomy will recur
Recommended surgical staging procedures

- Peritoneal washings
- Total abdominal hysterectomy and bilateral salpingo-oophorectomy
  (Unilateral salpingo-oophorectomy may be appropriate for selected patients with
  Stage IA disease who desire to defer definitive surgery until completion of
  childbearing.)
- Infracolic omentectomy
- Pelvic and para-aortic lymph-node sampling
- Peritoneal biopsies from:
  - cul-de-sac
  - rectal and bladder serosa
  - right and left pelvic sidewalls
  - right and left paracolic gutters
  - right and left diaphragms
  - any adhesions
# Results of restaging laparotomies in women with apparent early stage ovarian carcinoma

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Number of patients</th>
<th>FIGO stage at initial surgery</th>
<th>% upstaged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagley 1973</td>
<td>5</td>
<td>I-II</td>
<td>60%</td>
</tr>
<tr>
<td>Young 1983</td>
<td>100</td>
<td>IA-IIB</td>
<td>31%</td>
</tr>
<tr>
<td>Helewa 1986</td>
<td>25</td>
<td>I</td>
<td>20-25%</td>
</tr>
<tr>
<td>Buchsbaum 1989</td>
<td>140</td>
<td>I-II</td>
<td>22.4%</td>
</tr>
<tr>
<td>Archer 1991</td>
<td>24</td>
<td>I-II</td>
<td>20.8%</td>
</tr>
<tr>
<td>Soper 1992</td>
<td>30</td>
<td>I-II</td>
<td>30%</td>
</tr>
<tr>
<td>Stier 1998</td>
<td>45</td>
<td>IA-IIB</td>
<td>16%</td>
</tr>
<tr>
<td>Leblanc 2000</td>
<td>28</td>
<td>I</td>
<td>21%</td>
</tr>
</tbody>
</table>
Survival by initial tumor size
Second look surgery: Why perform it?

- **Contra**
  - Recurrence rates of 50 % after negative second look surgery
  - Absence of proven salvage therapy
  - Lack of demonstrable survival benefit

- **Pro**
  - No proven alternative surveillance techniques (CT, Ca125, etc.)
  - Possible survival benefit of secondary cytoreduction
  - Possible long term survival benefit for patients undergoing second line chemotherapy with minimal residual disease.
Survival by performance of second look

- Second look performed: 78 patients
- Second look refused: 29 patients
- \( P = 0.0028 \)
Survival by outcome of second look

Cumulative Survival

Survival (Months)
Potential of *In Vivo* Fluorescence

- **Staging laparotomy**

- **Second Look**
AIMS

- To evaluate *photodetection* of ovarian cancer peritoneal implants in the animal model
- To study pharmacokinetics of the photosensitizer precursor aminolevulinic acid (ALA)
- To evaluate *photodetection* of ovarian cancer peritoneal implants in patients
- To analyse toxicity of ALA *photodynamic therapy* (PDT) in the animal model
Enhanced diagnosis through photodetection

- *Photodetection* of ovarian cancer peritoneal implants in the animal model
- Determination of the best Photosensitizer
- *Photodetection* of ovarian cancer peritoneal implants in ovarian cancer patients
NuTu-19 Ovarian Cancer Animal Model

- **Cell line** - NuTu-19 - Spontaneous mutation
- **Histology** - Poorly differentiated ovarian adenocarcinoma with papillary features
- **Growth pattern** - I.P. serosal nodules with local tissue invasion (omentum, diaphragm, liver, peritoneum)
- **Malignant ascites** - average vol. 50-70ml in 6 weeks
- **Survival** - 106 cells I.P are 100% fatal, mean survival of 50 days
- **Non-immunogenic tumor developed in an immunocompetent host**

Rose et al AJOG 9/96
Epithelial ovarian cancer PDD in NuTu-19 rat model

8mM h-ALA IV prior to photodetection 2 hours later
Light micrographs (A) and fluorescence (B) of a peritoneal nodule (size < 0.5 mm) 6 hr after ip ALA administration. Magnification (C) of the peritoneal serosa (boxed area in B) showing a thin layer of tumor matching with the fluorescence.

Numbers of metastases detected with white and blue light detection for different concentrations of h-ALA and ALA

<table>
<thead>
<tr>
<th>Concentration [mM]</th>
<th>Time after inst.</th>
<th>White light</th>
<th>Bluelight</th>
<th>Ratio</th>
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<tbody>
<tr>
<td>4</td>
<td>2.5</td>
<td>9</td>
<td>19</td>
<td>2.1</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
<td>0</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>2.0</td>
<td>21</td>
<td>37</td>
<td>1.8</td>
</tr>
<tr>
<td>8</td>
<td>2.0</td>
<td>36</td>
<td>57</td>
<td>1.6</td>
</tr>
<tr>
<td>8</td>
<td>2.0</td>
<td>13</td>
<td>29</td>
<td>2.2</td>
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<tr>
<td>8</td>
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<td>4</td>
<td>24</td>
<td>6</td>
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<td>8</td>
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<td>2.7</td>
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<td>12</td>
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<td>9</td>
<td>25</td>
<td>2.8</td>
</tr>
<tr>
<td>20</td>
<td>2.0</td>
<td>10</td>
<td>16</td>
<td>1.6</td>
</tr>
<tr>
<td>8 (ALA)</td>
<td>2.0</td>
<td>10</td>
<td>16</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Lüdicke F et al, Britsh J Cancer
Human Epithelial Ovarian cancer PDD

10mg/ml ALA applied topically prior to photodetection

Stage III-IV ovarian + 6-8 cycles taxoid platinum chemotherapy

Clinical complete response

Second-look operation with white light and PDD.

Neg. second-look operation with white and PDD.

Pos. second-look operation with white light or PDD

Retrospective Data
No second-look

Lesions < 1/2 cm via white or PDD or microscopic disease

Second-line chemotherapy

Survival data.

Macroscopic lesions > 1/2 cm via white or PDD

Second line chemotherapy.

Survival data.

Retrospective survival data.
CONCLUSIONS

• Photodetection has been shown to be efficient in the animal model and feasible in patients

• Photodetection of ovarian cancer peritoneal implants, not visible by other methods, is a conceivable goal for the future

• The impact on survival has to be demonstrated in further studies
“The facts remains that a large number of patients are being treated almost to the point of “cure” and an additional stroke of some sort is needed.”

(DiSaia, Clinical Gynecological Oncology, Mosby-Year Book, 1997)
Light micrographs (A) and fluorescence (B) of small intestine 6 hr after ip ALA

AIMS

- Proof of principle of gene based photodynamic therapy of the peritoneal cavity after IP administration of ALA-S virus (establishment of a stable NuTu 19 ALA-S mutant cell line)
Problems in gene therapy

- Transfection, transduction rate
- Side effects
- Tissue penetration
- Immune reaction
- Specificity
Results

• Pp IX production in the NuTu-19 ovarian cancer cell line after ALA and ALA-S mutant adenovirus application

• Toxicity (cell killing) of ALA, ALA-S virus and LacZ adenovirus in NuTu-19 cells

• Transduction rate of GFP adenovirus (CMV) in NuTu cells and in control cells (293T)
Perspective

- Proof of efficient photodynamic therapy in the animal model after I.P ALA-S virus administration, impact on survival
- Increase transduction rate
- Achieve cancer specific expression of the transgene
PDT of cervical intraepithelial neoplasia

• Rationale
• Introduction
• Study design
• Material and Methods
• Results
Aim

• Determine if h-ALA is selectively absorbed by dysplastic cells at various times after topical application (5min to 7 hours)
Rationale

• Increasing incidence of cervical precancerous lesions in younger women
• Treatment of precancer of the cervix (conization) is an invasive procedure with its peri-operative risk and potential long term risk for fertility
Treatment of CIN: Excisional methods

- Cold knife conisation
- Loop electrosurgical excision procedure (LEEP)
Treatment of CIN: Ablative methods

- Cryotherapy
- Laser vaporisation
- Photodynamic therapy
Advantages to treat CIN with PDT

• Outpatient clinic
• Specificity (drug, light)
• Tailored to the shape of the cervix
• No stromal destruction (stenosis, cervix insufficiency)
• Cell death by apoptosis (no inflammation, no scaring)
• Specific HPV destruction (tetrapyrrol)
• Repeatable
Representative spatial distribution of 5-ALA induced porphyrin fluorescence related tissue type

Material and methods

• Phase I clinical trial involving 30 non-pregnant women with already biopsy-proven CIN 1-3
• Application of a 0.5 % h-ALA
• Random biopsies at time points ranging from 5min to 7 hours
• Image analysis on frozen tissue sections using Zeiss Axiophot image analysis system
Topical application of h-ALA

- Rinsing the cervix with physiological NaCl
- A solution of h-ALA of 0.5% is applied topically on the cervix with help of a gauze sponge and cervical cup
- 5 biopsies are taken in dimmed light before performing conization
Fluorescence image and white light image of the cervix uteri after the application of 3% acetic acid. Application of 10mg h-ALA in 10ml 0.9% NaCl solution on the cervix during 3 hrs.
Cin III 20x (exocol), h-ALA 0,5%, 75 min

E- epithelium, LP-lamina propria
Cin II (exocol) 10X, h-ALA 0,5%, 30 min

E- epithelium, LP-lamina propria

Départements de Pathologie Clinique et de Gynécologie et Obstétrique HUG & EPFL, mai 2002
Cin II 20X (exocol), h-ALA 0,5%, 5min.

E- epithelium, LP-lamina propria

Départements de Pathologie Clinique et de Gynécologie et Obstétrique HUG & EPFL, mai 2002
Patients with high-grade cervical intraepithelial neoplasia (HSIL, CIN II-III,) in whom it is planned to perform a conisation.

Pretreatment with topically applied hexyl-Aminolevulinic acid. Formulation: 0.5% h-ALA creme. Application time: 30, 60, 120, 180 minutes.

Photodynamic treatment with non-thermal laser (635 nm; 200mW/cm²; 100 J/cm²). Application time: 10 minutes.

One month later.

Conisation.

Histopathological examination.

Evaluation of the result of PDT.

Long-term follow-up.