Fluorescence Detection of Ovarian Cancer in the NuTu-19 Epithelial Ovarian Cancer Animal Model using Aminolaevulinic Acid hexylester

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Fluorescence of Peritoneal Nodules 2.0 hours after i.p. injection
<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>5-year survival Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I</td>
<td>Growth limited to the ovaries</td>
<td>61</td>
</tr>
<tr>
<td>Ia</td>
<td>One ovary involved</td>
<td>65</td>
</tr>
<tr>
<td>Ib</td>
<td>Both ovaries involved</td>
<td>52</td>
</tr>
<tr>
<td>Ic</td>
<td>Ascites present, or positive peritoneal washing, tumor on the surface of the ovary</td>
<td></td>
</tr>
<tr>
<td>2. II</td>
<td>Growth limited to pelvis</td>
<td>40</td>
</tr>
<tr>
<td>Iia</td>
<td>Extension to the uterus and the tubes</td>
<td>60</td>
</tr>
<tr>
<td>Iib</td>
<td>Extension to other pelvic tissues</td>
<td>38</td>
</tr>
<tr>
<td>Iic</td>
<td>Like Ic</td>
<td></td>
</tr>
<tr>
<td>3. III</td>
<td>Growth extending to abdominal cavity, including peritoneal surface and omentum</td>
<td>5</td>
</tr>
<tr>
<td>IIIa</td>
<td>Microscopic abdominal implants, negative nodes</td>
<td></td>
</tr>
<tr>
<td>IIIb</td>
<td>Macroscopic abdominal implants, &lt; 2 cm, negative nodes</td>
<td></td>
</tr>
<tr>
<td>IIIc</td>
<td>Abdominal implants &gt; 2 cm and/or positive nodes</td>
<td></td>
</tr>
<tr>
<td>4. IV</td>
<td>Metastases to distant sites (positive pleural cytology, parenchymal liver metastasis)</td>
<td>3</td>
</tr>
</tbody>
</table>

The 5-year survival rate of ovarian Cancer in Geneva
## 5-year cumulative lethality rate of gynecologic malignancies in Geneva

<table>
<thead>
<tr>
<th>Interval</th>
<th>Cervix uteri</th>
<th>Corpus Uteri</th>
<th>Ovary</th>
<th>Other genital organs</th>
<th>Breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1(^{st})</td>
<td>15.3%</td>
<td>16.3%</td>
<td>43.7%</td>
<td>28.0%</td>
<td>9.1%</td>
</tr>
<tr>
<td>0-2(^{nd})</td>
<td>27.3%</td>
<td>25.2%</td>
<td>59.9%</td>
<td>38.7%</td>
<td>16.1%</td>
</tr>
<tr>
<td>0-3(^{th})</td>
<td>33.3%</td>
<td>30.6%</td>
<td>67.8%</td>
<td>40.9%</td>
<td>23.7%</td>
</tr>
<tr>
<td>0-4(^{th})</td>
<td>38.2%</td>
<td>34.5%</td>
<td>71.1%</td>
<td>47.3%</td>
<td>30.2%</td>
</tr>
<tr>
<td>0-5(^{th})</td>
<td>42.3%</td>
<td>37.6%</td>
<td>72.2%</td>
<td>52.7%</td>
<td>35.1%</td>
</tr>
</tbody>
</table>

Data from Geneva 1970-1994
Comparative rate (Europe) per 100'000 population per year

Year specific mortality curve of genital malignancies, Geneva 1970-1994
Pp IX Spectrum measured on Peritoneal Nodule
Scattering and refraction of light

Rayleigh scattering

Mie scattering

Refraction
Light propagation in absorption dominated and in scattering dominated tissues. Small solid and open circles represent absorbers and scatterers, respectively. Larger open circles represent target molecules.
Absorption of water, melanin (broken line) and oxyhemoglobin (HbO₂) (dotted line)
Penetration depth of light in tissue in relation to the wavelength
Excitation of photosensitizer and singlet oxygen generation

Excited singlet photosensitizer → Intersystem crossing → Excited triplet photosensitizer → Excitation of singlet oxygen → Optically forbidden → Singlet oxygen → Optically forbidden → Target cell

Optical excitation → Fluorescence → Singlet photosensitizer

Cytotoxic action
Chemical structure of 5-ALA and PpIX. Me represents methyl group
Feedback control

Exogenous 5-ALA

Glycine succinyl CoA

ALA-S

ALA synthase

5-ALA

AL hydratase

ALA D

2H₂O

PBG
dehydrolase

PBG

Porphobilinogen

dehaminase

4NH₃

H₂O

Hydroxymethylbilane

Uroporphyrinogen III synthase

Uroporphyrinogen III
decarboxylase

Protoporphyrinogen IX oxidase

Protoporphyrinogen IX

Protochlorophyllin oxidase

Protochlorophyllin IX

Heme

2H⁺

Fe²⁺

Ferrochelatase

Mitochondrion

Enzyme activity in neoplastic tissue

increased

decreased
Absorption (blue line) and fluorescence (pink line) spectrum of PpIX solved in DMSO. Values of absorption and fluorescence do not correspond to each other.
Set up of the optical fiber based spectrofluorometer
Drug Injection Data: the table shows the total number of rats, the injected drug, the drug dose and the time delay between injection and measurement

<table>
<thead>
<tr>
<th>Number of Rats</th>
<th>Drug</th>
<th>Concentration</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>h-ALA</td>
<td>4mM</td>
<td>2.0 h</td>
</tr>
<tr>
<td>2</td>
<td>h-ALA</td>
<td>4mM</td>
<td>2.5 h</td>
</tr>
<tr>
<td>1</td>
<td>h-ALA</td>
<td>8mM</td>
<td>0.5 h</td>
</tr>
<tr>
<td>1</td>
<td>h-ALA</td>
<td>8mM</td>
<td>1.0 h</td>
</tr>
<tr>
<td>1*</td>
<td>h-ALA</td>
<td>8mM</td>
<td>1.5 h</td>
</tr>
<tr>
<td>4</td>
<td>h-ALA</td>
<td>8mM</td>
<td>2.0 h</td>
</tr>
<tr>
<td>2</td>
<td>h-ALA</td>
<td>12mM</td>
<td>2.0 h</td>
</tr>
<tr>
<td>2*</td>
<td>h-ALA</td>
<td>12mM</td>
<td>2.5 h</td>
</tr>
<tr>
<td>2*</td>
<td>h-ALA</td>
<td>20mM</td>
<td>2.0 h</td>
</tr>
<tr>
<td>2</td>
<td>ALA</td>
<td>8mM</td>
<td>2.0 h</td>
</tr>
</tbody>
</table>
Reference signal

Optical fiber in measuring position on the omentum; the red fluorescing tissue at the “10-o’clock” position shows a part of the fluorescing intestine
D-Light Inspection

- After spectrometric measurements the abdomen was inspected with the Storz D-Light system. The quantities and settlings of the metastases that could be observed in the white and blue light mode, respectively, were noted. Moreover pictures of the metastases in white light and blue light were recorded by the video system.
Fluorescence metastases in dependence on drug concentration

Fluorescence of peritoneal nodules in dependence on drug concentration 2 to 2.5 h after drug injection; if not noted otherwise values give concentration of h-ALA, n depicts the number of rats.
Fluorescence emission of healthy (blue) and nodular tissue (violet)

Physical contrast C of nodular to healthy tissue for different concentrations of ALA and h-ALA
Blue and white light mode images of peritoneal nodules taken 2.0 hours after i.p. injection of 8mM (A) and 20mM (B) h-ALA, respectively. The blue light image B shows clearly the high red fluorescence of the healthy tissue that turned out to be difficult for detection of very small nodules. On the other hand, fluorescence of the big nodule in picture B is higher than in the nodules shown in picture A.
Time-dependence of nodule fluorescence and fluorescence of healthy peritoneal tissue (dotted line) emission in rats injected with 8 mM h-ALA

Information on time-dependence of the generation of PpIX was obtained from 7 rats injected with 8mM h-ALA. Fluorescence emission from the nodules and healthy tissue was acquired by spectrometer and the peritoneal sites were inspected with the D-Light system.
Blue and white light mode images of peritoneal nodules in rats sensitized with 8mM h-ALA taken at 0.5 (A), 1.0 (B) and 2.0 (C) hours after i.p. injection respectively. The increase of nodule fluorescence with time is apparent. Fluorescence achieved with ALA with a time delay of 2 h is comparable with that achieved with the same dose h-ALA after 0.5 h (D).
Numbers of metastases detected with white and blue light detection for different concentrations of h-ALA and ALA. The ratio of nodules detected in blue light to those detected with white light exceeds 1.6 for all drugs and all concentrations.
Images of peritoneal metastases in blue and white light mode: image A shows a lesion that is only visible in the blue light mode, but not with white light (position marked by a circle), (8mM h-ALA after 2.0h). Image B shows three lesions visible in blue and white light (big circle) and one only detectable by fluorescence (small circle) (20mM, 2.0h)
Small intestine

Blue and white light images of the small intestine. The human intestine shows no native PpIX fluorescence that was observed in the digestive organs of the rats.
Conclusion

- The photosensitizer precursor Aminolaevulinic Acid hexylester (h-ALA) is suitable to detect micrometastases by means of photodiagnosis in the ovarian cancer animal model. Administered at the same dosage of 8 mmol and applied during the same time interval h-ALA results in higher PPIX fluorescence emission than its counterpart ALA. The clinical impact of these findings remain to be shown.
Fluorescence of Pax, Theil and Mouth Mucosa 2 hours after i.p. injection

Fluorescence emission [a.u.]

- Tail
- Paw
- Mouth

8mM ALA
8mM ALA
8mM h-ALA
20mM h-ALA
20mM h-ALA
Cervical cancer in situ tendency in Geneva

Tumor Registry of Geneva, May 1996
Block diagram of the Cancer Photodetection apparatus
Glycine + Succinyl CoA

5-Aminolevulinic acid synthetase (Feedback control)

Ferrochelatase + Fe^{2+}

Protoporphyrin IX

Protoporphyrinogen oxidase

5-Aminolevulinic acid

Heme

Protoporphyrinogen IX

5-Aminolevulinic acid dehydrase

Cytosol

Coproporphyrinogen oxidase

5-Aminolevulinic acid

Coproporphyrinogen III

Coproporphyrinogen deaminase

Protoporphyrinogen I

Protoporphyrinogen III

Uroporphyrinogen I

Porphobilinogen

Uroporphyrinogen III

Protoporphyrinogen III

Coproporphyrinogen III

Coproporphyrin I

Uroporphyrin I

Uroporphyrin III

Coproporphyrin III

mitochondrion
Cervicoscopy after topical ALA application

Cervix white light examination

Cervix fluorescence under TDP (green-bleu)
HE and Fluorescence microscopy

HE colored cross section of the cervix with CIN lesion

Fluorescence microscopy cross section of the cervix with low-grade CIN lesion
Surface illumination of 30 mm long distributor (in air)
Light distributor for PDT in the cervix

- Polymer Optical Fiber Ø 1mm
- Stainless steel tube
- Fiber jacket
- Teflon tube
- Delrin
- Mirror
- Light diffuser

Dimensions:
- 200 mm
- 10 mm
- 23, 33, or 43 mm
- Ø 3.5 mm
- Ø 10 mm
Instrumentation set-up for the fluorescence imaging tumor depth profiling
Principle of fluorescence imaging tumor depth profiling:
Homogenous excitation of the fluorochrome concentrated in the tumoral tissue at three different wavelengths, corresponding to the absorption maxima of the fluorochrome (417, 514, 633 nm).
Detection at the emission maxima (650-720) nm.
5-Aminolevulinic acid and PPIX concentrations after oral administration (40 mg/kg b.w.). [Rick et al. 1997]
Fluorescence intensity after oral administration of 5-aminolevulinic acid (40 mg/kg). [Rick et al. 1997]