
Synthes Award 2007
10 Steps to a more radical Tumor Removal in GBM Patients

A in vitro study of interactions and apoptosis in the presence of 5ALA

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PIX Fluorescence in malignant Glioma surgery

- Diagnostic

<table>
<thead>
<tr>
<th>Fluorescence +</th>
<th>Specificity: 17/17+3 = 0.85</th>
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<td>Sensitivity: 31/31+10 = 0.76</td>
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<tr>
<th>Fluorescence ++</th>
<th>Specificity: 1.0</th>
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<tr>
<td></td>
<td>Sensitivity: 42/42+1 = 0.98</td>
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PIX Fluorescence in malignant Glioma surgery

Therapy:
The Quest

- Do commonly administered antiepileptic and chemotherapeutic drugs interact with PIX accumulation in GBM?

- What is the optimal light source and exposure time for a selective therapy in PDT with GBM?
GBM cell lines

- **U373 MG**
  - well known specifications

- **U251 MG**
  - well known specifications

- **SNB 19**
  - laminin slows growth and motility of cells

- **U87 MG**
  - wild p53 less prone to apoptosis

Drugs

- **Phenytoin** Phenydan ®
- **Levetiracetam** Keppra ®
- **Lamotrigin** Lamictal ®
- **Phenobarbital** Luminal ®
- **Carbamazepin** Tegretol ®
- **Temozolomid** Temodal ®
- **Lomustin** Cecenu ®

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Preliminary study
Step 1-5

1. Do all GBM cell lines accumulate PIX in the presence of 5ALA?

2. What is the \([\text{PIX}]_{\text{max}}\) per individual GBM cell?

3. What is the \([\text{PIX}]_{\text{max}}\) corresponding fluorescence intensity per cell?

4. Cell lines with no visibly discernable fluorescence (eg. Medulloblastoma), what is their \([\text{PIX}]_{\text{max}}\) and corresponding fluorescence intensity?

5. GBM and Medulloblastoma in co culture, how accurate can we define the borderline by fluorescence
Growth curve of four different glioblastoma cell lines
Main Study
Step 6 and 7
drug related interaction

• Step 6: Dose response analysis, Culture vs. Drug; MTT Assay
  1. Drug related mitochondrial inactivity

• Step 7: Dose response analysis, Culture, Drug, 5ALA; MTT and FL
  1. 5ALA/ drug MTT – drug MTT
  2. 5ALA related mitochondrial inactivity
  3. Drug related fluorescence intensity
Dose Response
MTT Assay

- U373 MG
- U251MG
- SNB19
- U87 MG
to
- Phenytoin,
  Levetiracetam,
  Lamotrigin,
  Phenobarbital,
  Carbamazepin and
  Temozolomide, Lomustin
each

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Dose Response
MTT and FL Assay

- U373 MG
- U251MG
- SNB19
- U87 MG
- to
- Phenytoin, Levetiracetam, Lamotrigin, Phenobarbital, Carbamazepin and Temozolomide, Lomustin
- each and
- 5 ALA

Response: MTT and PIX

Dose-Response curves: Determination of IC50 after having incubated the glioblastoma cell lines U-373MG and U251 MG with ALA for 48 hours.
Dose-Response curves: Determination of IC$_{50}$ after having incubated the glioblastoma cell lines U-373MG and U251 MG with ALA for 48 hours

Cell line
- U-373 MG

IC$_{50}$: 3.8087 mg/ml ± 0.109

Cell line
- U251 MG

IC$_{50}$ = 2.3033 mg/mL ± 0.124
Main Study
Step 8, 9 and 10
Photodynamic Therapy

- **Step 8**: Dose response analysis, Culture and Co Culture, 5ALA, exposure time, MTT Assay

- **Step 9**: PCR and immunostaining for the detection of activated apoptosis genes as proof of successful PDT

- **Step 10**: PDT in 3D GBM culture, evidence of BIRC 3, PARP, Caspase 3 activity to define penetration depth
Duration of illumination for >90% cell death

BIRC 3, PARP, Caspase 3

Y83-77
What we learn

- Influence of common antiepileptic and chemotherapeutic medication on PIX Fluorescence intensity in GBM

- Optimal light source and exposure time for PIX PDT in GBM for max cell death and specificity and exposure depth in malignant Gliomas

Perspectives

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