A Method to Determine the Reversibility of Inhibition for Glutamate Carboxypeptidase II Inhibitors using BioLayer Interferometry

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March 6, 2014
Prostate Cancer: Background

- Most commonly diagnosed cancer among US men and accounts for the second leading cause of death

- 186,320 American men were diagnosed and 28,660 succumbed to the disease

- 1 in 6 men will be diagnosed in their lifetime
Prostate Cancer: Diagnosis and Treatment

• Current diagnosis methods
  – Irregular digital rectal exam (DRE)
  – Elevated prostate specific antigen (PSA) level
    • Does not stage prostate cancer
    • Sensitivity limits diagnosis before it metastasis
  – Prostascint
    • Radiolabeled murine monoclonal antibody
    • Image metastatic prostate cancer
    • Targets an intracellular epitope of PSMA only accessible in dead, dying or apoptotic cells

• Current treatment plans
  – Prostatectomy
  – Radiation, hormone, and chemotherapy for more advanced stage
  – Once metastases to the bone, improve quality of life
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There is a need for early diagnostic tools
PSMA: Background

- Prostate specific membrane antigen (PSMA)—established biomarker for prostate cancer
- Overexpressed on surface of prostate cancer cells
- Internalization is induced by antibody or ligand binding and mediated via clathrin-coated pits
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Ideal target for delivery of prostate cancer imaging and therapeutic agents
Reversibility of Inhibition on Uptake and Internalization

\[
\begin{align*}
\text{X=NH: } & \quad 99\text{mTc(CO)}_3\text{-DPA-DBCO-PEG}_4\text{-CTT-54} \\
\text{X=O: } & \quad 99\text{mTc(CO)}_3\text{-DPA-DBCO-PEG}_4\text{-CTT-54.2}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Time (hr)</th>
<th>% Uptake$^a$</th>
<th>% Internalization$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$99\text{mTc(CO)}_3\text{-DPA-DBCO-PEG}_4\text{-CTT-54}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LNCaP</td>
<td>0.5</td>
<td>7.95 (±0.35)</td>
<td>42 (±1.7)</td>
</tr>
<tr>
<td>LNCaP</td>
<td>2.0</td>
<td>19.06 (±0.65)</td>
<td>51 (±5.4)</td>
</tr>
<tr>
<td>LNCaP</td>
<td>4.0</td>
<td>24.85 (±0.41)</td>
<td>52 (±3.0)</td>
</tr>
<tr>
<td>PC-3</td>
<td>0.5</td>
<td>0.33 (±0.07)</td>
<td>N/A</td>
</tr>
<tr>
<td>PC-3</td>
<td>2.0</td>
<td>0.89 (±0.28)</td>
<td>N/A</td>
</tr>
<tr>
<td>PC-3</td>
<td>4.0</td>
<td>1.28 (±0.22)</td>
<td>N/A</td>
</tr>
<tr>
<td>$99\text{mTc(CO)}_3\text{-DPA-DBCO-PEG}_4\text{-CTT-54.2}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LNCaP</td>
<td>0.5</td>
<td>1.46 (±0.66)</td>
<td>40 (±3.9)</td>
</tr>
<tr>
<td>LNCaP</td>
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<td>4.79 (±0.19)</td>
<td>30 (±3.1)</td>
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<tr>
<td>LNCaP</td>
<td>4.0</td>
<td>9.16 (±0.26)</td>
<td>27 (±4.2)</td>
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<tr>
<td>PC-3</td>
<td>0.5</td>
<td>0.35 (±0.20)</td>
<td>N/A</td>
</tr>
<tr>
<td>PC-3</td>
<td>2.0</td>
<td>0.21 (±0.09)</td>
<td>N/A</td>
</tr>
<tr>
<td>PC-3</td>
<td>4.0</td>
<td>0.13 (±0.04)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

$^a$Standard deviation in parentheses.

HPLC Method to Determine Reversibility of Inhibition

Rapid dilution of enzyme-inhibitor complex to monitor recovery

Incubate
100x enzyme
10x IC50 inhibitor

100X dilution
w/ sat.
[substrate]

Incubate
1x enzyme
1/10x IC50 inhibitor
10 uM substrate

Monitor product formation by HPLC
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10 uM substrate

Results of Rapid Dilution Assay

Biochemistry 2008, 47, 12658–12660
Research Objective:

Develop a more convenient method to evaluate reversibility of inhibition

Criteria:
- Quick
- Easy
- Preferably in real-time
BLI Assay

BLI Assay

A) Strepavidin coated biosensor tips

B) Strepavidin coated biosensor tips

C) Strepavidin coated biosensor tips
BLI Assay: Results

R= Biotin-PEG12 (Biotin-PEG12-CTT54)

R= Biotin-PEG12 (Biotin-PEG12-CTT54.2)

R= Biotin-PEG12 (Biotin-PEG12-Lys-Glu urea)
Summary: BLI Assay

• Developed a quick, easy, real-time assay

• Demonstrated a protein can be immobilized to the biosensor tip via protein-small molecule inhibitor interaction

• BLI can be used to determine the reversibility of PSMA inhibitors

• Results from BLI study are consistent with HPLC reversibility study
  – CTT54 is a pseudo-irreversible inhibitor
  – CTT54.2 is a slow reversible inhibitor
  – Urea compound is rapidly reversible
Alternative BLI Assay

- Develop BLI assay that does not require a biotinylated inhibitor
  - Advantage: Does not require biotinylation of each inhibitor to determine reversibility of inhibition
  - Requires biotinylated PSMA
- Reversibility of inhibition against biotinylated-PSMA for known inhibitors was determined with traditional HPLC method
Unusual Observations: PMPA
Unusual Observations: CTT54
Unusual Observations: CTT54.2
Closer Examination of PMPA dilution

Blue: 100 uM PMPA; Red: 1 uM PMPA; Turquoise: 0.01 uM PMPA
Closer Examination of PMPA dilution

Blue: 100 uM PMPA; Red: 1 uM PMPA; Turquoise: 0.01 uM PMPA

[Diagram showing PMPA concentration over time with different colors representing different concentrations.]
Closer Examination of CTT54 Dilutions

Blue: 100 uM CTT 54; Red: 1.0 uM CTT 54; Turquoise: 0.1 uM CTT 54;
Closer Examination of CTT54 Dilutions

Blue: 100 uM CTT 54; Red: 1.0 uM CTT 54; Turquoise: 0.1 uM CTT 54;

![Graph showing dilutions and chemical structure with text: nanomolar IC50 Irreversible.]

Low [LW54] → High [LW54] → Dissociation
Closer Examination of CTT54.2 Dilutions

Blue: 100 uM PO-LW54; Red: 1.0 uM PO-LW54; Turquoise: 0.1 uM PO-LW54; Green: 0.01 uM PO-LW54
Closer Examination of CTT54.2 Dilutions

Blue: 100 uM PO-LW54; Red: 1.0 uM PO-LW54; Turquoise: 0.1 uM PO-LW54; Green: 0.01 uM PO-LW54

nanomolar IC_{50}
Slowly Reversible
Summary of Unusual Observations

- Observed binding to individual subunits and effects of different types of inhibitors (irreversible, slowly-reversible, reversible)
Summary of BLI Assay

• BLI can be used to determine the reversibility of PSMA inhibitors

• Results from BLI study are consistent with HPLC reversibility study
  – CTT54 is a irreversible inhibitor
  – CTT54.2 is a slow reversible inhibitor
  – PMPA is rapidly reversible
Acknowledgment

Washington State University
• Clifford E. Berkman and the Berkman Group
  – Wenye Deng
• Jeff Jones

Collaborator
• Cyril Barinka (Academy of Sciences of the Czech Republic)

Funding
NCI and NIAID