Cytotoxicity Evaluation of New Drug Delivery Vehicles

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Cancer

- Rapid creation of cells that grow beyond their usual boundaries
- Can invade and spread to different parts of the body
- Various treatments – surgery, radiation, chemotherapy

Figure 1. Cancer Metastasis.
(http://www.nature.com/nrc/journal/v3/n1/fig_tab/nrc967_F1.html)
Chemotherapy

- Interrupts cancer cell growth through the application of cytotoxic substances

- Drugs bind to DNA via
  - Intercalation i.e. planar aromatic molecules slot in between the DNA base pairs.
  - Groove binding i.e. small aromatic molecules bind to the minor or major grooves.
  - DNA alkylation i.e. the forms direct covalent bonds to one of the DNA bases.
Chemotherapy

• 6 main groups:
  – Alkylating agents.
  – Plant alkaloids.
  – Synthetic drugs.
  – Hormones and steroids.
  – Biological Materials.
  – Antimetabolites.

• But
  – high doses needed
  – may also affect normal cells with severe side effects
    – hair loss, nausea, joint pain etc
Drug Delivery Systems

Drug delivery systems are a solution to disadvantages of chemotherapy

- Recognise cancer cells
- Specifically target these cells
- Deliver the therapeutic dose
- Involve a Carrier, a Ligand and the Drug
Drug Delivery Systems

- Involve molecules capable of carrying a drug to a specific locale in the organism eg Cyclodextrins

Figure 2. Tumor targeting and endocytosis.
(winshipcancer.emory.edu/SPORE-ed/project4.htm)
Molecular Markers

- Molecular Markers are the targets eg Folate Receptors (FR) are commonly over-expressed in cancer cells
- Ligand is attracted to the molecular marker eg folate

Figure 3. Targeting cancer cell through molecular markers. (http://www.genengnews.com/gen-articles/molecular-imaging-driving-development/4949/?page=2)
Figure 3. Proposed chemical structure of targeted drug delivery system CDEnFA:MTX.
Folate Receptors

- Over-expressed in cancer cells e.g. lung, breast, cervical and bladder [1].
- Proteins located on cellular membranes.
- Bind and transport folic acid into the cells for synthesis of DNA

**Figure 4.** Tumour targeting and Endocytosis [2].


Cyclodextrins

- CDs are cyclic oligosacharides with α-1-4 glycosidic bonds
- α-CD = 6 glucose, β-CD = 7 glucose, γ-CD = 8 glucose

**Figure 5.** Structure of cyclodextrin
Cyclodextrins

- Hydrophilic exterior due to −OH groups
- Hydrophobic cavity due to H and −O− groups

Figure 5. Structure of cyclodextrin
(http://www.pharmatutor.org/articles/colon-drug-delivery-system-recent-conventional-and-novel-approach?page=0.2)
Cyclodextrins

- CDs have the ability to form inclusion complexes in aqueous solution, in which molecules of compatible dimensions are included within the cavity.

**Figure 6.** Cyclodextrin cavity and inclusion.  
Methotrexate (MTX)

- Methotrexate is a competitive inhibitor of folic acid, and therefore, it acts to prevent folic acid metabolism, inhibiting the synthesis of DNA.

![Chemical structure of MTX](withfriendship.com/user/boss/methotrexate.php)
Synthesis

6-o-monotosyl-6-deoxy-β-cyclodextrin (CDTs): Yield: 42.9%
melting point: 162-168.7°C (Lit.val. 161-162°C)[4].

6-deoxy-6-[1-(2-amino)ethylamino]-β-cyclodextrin (CDEn): Yield: 76.5%
melting point: 236°C (Lit.val 268-271°C)[4].

CDEn-FA delivery system. Yield: 60% (based on hydrated materials) melting point: 254°C[6].

Final product drug delivery system CDEn-FA was analysed by a number of spectroscopic techniques as:

- FTIR
- Raman
- MS
- NMR

Synthesis of Inclusion Complex

Figure 8. Proposed chemical structure of targeted drug delivery system CDEnFA:MTX.
Analysis of inclusion complex CDEn-FA:MTX

Figure 9. $^1$H NMR in D$_2$O of (a) CDEn-FA and (b) CDEn-FA:MTX 1:1 ratio.

Table 1. $^1$H NMR data of free CDEn-FA and inclusion complex CDEn-FA:MTX in D$_2$O.

<table>
<thead>
<tr>
<th>Proton</th>
<th>Free CD (ppm)</th>
<th>CD:MTX (ppm)</th>
<th>$\Delta$ ppm</th>
<th>Inclusion with CTZ (ppm)[7]</th>
<th>Inclusion with 2-NOH-6 (ppm)[8]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3</td>
<td>3.820</td>
<td>3.666</td>
<td>0.154</td>
<td>0.438</td>
<td>0.220</td>
</tr>
<tr>
<td>H5, H6</td>
<td>3.734</td>
<td>3.570</td>
<td>0.164</td>
<td>0.206</td>
<td>0.220</td>
</tr>
<tr>
<td>H2</td>
<td>3.524</td>
<td>3.394</td>
<td>0.130</td>
<td>0.055</td>
<td>0.085</td>
</tr>
<tr>
<td>H4</td>
<td>3.463</td>
<td>3.324</td>
<td>0.139</td>
<td>0.046</td>
<td>0.065</td>
</tr>
</tbody>
</table>

Cytotoxicity

- Cytotoxicity is the degree to which something is toxic to living cells
- Various assays – Alamar Blue, Neutral Red, MTT
- Control = DMSO, Standard = Cisplatin
MTT assay

- Colorimetric assay for assessing cell viability
- In living cells, enzymes reduce the yellow tetrazolium salt MTT to its insoluble formazan, which has a purple colour
- Measurement of absorption in 96 well plate at 595 nm

Figure 10. Reduction of MTT formation of formazan dye.
(http://www.biotek.com/resources/articles/quantification-cell-viability-epoch.html)
Cell Lines and Protocol

- **HeLa** cervical cancer cell line taken from patient Henrietta Lacks in 1951.
- **MCF-7** (Michigan Cancer Foundation) breast cancer cell line isolated from 69 years old Caucasian woman in 1970.
- **A549** lung cancer cell line was first developed in 1972.

  All of which over-express folate receptors!

- **BEAS-2B** human normal lung cell line.
- Drug concentration (50 μM – 0.5 μM).
- Time of drug exposure 24 hours
MCF-7 (breast cancer) Cytotoxicity

HeLa (cervical cancer) Cytotoxicity
### A549 (lung cancer) Cytotoxicity

- **Drug concentration (μM)**: 0, 0.5, 1, 5, 10, 50
- **% Cell viability**

### BEAS-2B (lung normal) Cytotoxicity

- **Drug concentration (μM)**: 0, 0.5, 1, 5, 10, 50
- **% Cell viability**
Cytotoxicity EC\textsubscript{50}

\textit{EC}_{50} \textit{is a half maximal effective concentration}

Table 2. EC\textsubscript{50} values

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CDEn-FA</th>
<th>MTX</th>
<th>CDEn-FA:MTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa (Cervical cancer)</td>
<td>1290.6±658 µM</td>
<td>82.4 ±4.5µM</td>
<td>67.6 ±7.8µM</td>
</tr>
<tr>
<td>MCF-7 (Breast cancer)</td>
<td>1316.4 ±760 µM</td>
<td>9.4±1.6 µM</td>
<td>1.5±0.7 µM</td>
</tr>
<tr>
<td>A549 (Lung cancer)</td>
<td>1119.0±558 µM</td>
<td>188.0±16.7 µM</td>
<td>140.0±11.4 µM</td>
</tr>
<tr>
<td>BEAS-2B (Lung normal)</td>
<td>1245.9±624 µM</td>
<td>106.3±11µM</td>
<td>430.9±63 µM</td>
</tr>
</tbody>
</table>
Conclusion

- CDEn-FA non toxic compared to anti-cancer agents.

- MTX toxicity is greater for the MCF-7 cell line than A549 or HeLa.

  Trend observed is:

  MCF-7(Breast) > HeLa (Cervical) > BEAS-2B (normal Lung) > A549 (Lung)

- Toxicity of MTX is greater with CDEn-FA for all cancer cell and four times less in normal cell lines.

  Trend observed is:

  MCF-7(Breast) > A549 (Lung) > HeLa (Cervical) > BEAS-2B (normal Lung)