Targeted Bioresponsive Nanoparticles

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2006 Particles Meeting
Orlando, Fl
May 14, 2006
Is there's plenty of room at the bottom?

Liposomes

100nm

Magnetic nanoparticles

10nm

Aspirin

1nm

1µm

Red blood cells

10µm

Dendrimers

Quantum dots

Nature Biotechnology 21, 1161 - 65 (2003); Science 294, 1901 - 3 (2001); Ian Wark Research Institute
Summary

• Liposomes & Doxil™

• Non-biodegradable dendrimers

• Biodegradable dendrimer
Lipid Structure

Hydrophilic Interface Hydrophobic
LIPOSOME GENERATION

Phospholipid Molecule + Water → Bilayer → Liposome
Time Line for Liposome Drug Carriers

- Biomembrane 1965
- Enzyme encapsulation 1971
- Pharmacokinetics mid 70’s
- Therapeutics late 70’s
- Clinical trials 80’s
- Initial approval early 90’s
- Doxil™ approval 96
Drug Concentrations at a Tumor Site Following I.V. Administration

- Free drug
- Carrier-associated drug
- Free drug released from carrier accumulated at tumor site
Structure of Doxil

Doxorubicin

Lipid Membrane (Phospholipid + Cholesterol)

Polyethylene Glycol

85-100 nm

F. Martin
Doxil Morphology

F. Martin
DOXIL Pharmacokinetics

Plasma concentration (ng/mL)

Time After Infusion (Days)

$T_{1/2} = 2.5\ d$

$T_{1/2} = 3.8\ d$

Metabolite appears in few days

F. Martin
Serial Gamma Scintigrams of KS Patient after Pegylated Liposomes Containing $^{111}$In-DTPA

F. Martin

4 hrs. 24 hrs. 48 hrs. 96 hrs.
What Might Further Improve Liposome Drug/Gene Delivery?

- Cell targeting
  - CD44 ligands
- Improved cytoplasmic delivery
  - pH sensitive endosomal escape
- Control of surface properties
  - Redox surface
Drug Carriers

- Limit drug entry into healthy tissue and cells
- Increase drug solubility, modify immunogenicity and enzymatic degradation
- Passively target drug to tumors via “leaky” vasculature (EPR effect)
  - Tumor accumulation enhanced as size increases to 500 nm

Dendritic Molecules for Drug Delivery

Highly branched

- Multiple end-groups for drug attachment
- “Surface” functionality can be tailored
- Tunable solubility
- Decreased flexibility

Well-defined, monodisperse < 10 nm

- Reproducible synthesis
- Reproducible pharmacokinetics

Lee et al., Designing Dendrimers for Biological Applications, Nature Biotech. 23, 1517, 2005.
POLYAMINES USED TO FORM DNA POLYPLEXES

Non-buffering
Poor gene transfer

Strongly buffering
Good gene transfer
‘PROTON SPONGE’ HYPOTHESIS

Low buffering DNA polyplex

High buffering DNA polyplex
STRATEGY TO MEASURE ORGANELLAR CHLORIDE

Requirements of Cl\(^{-}\) sensor:

*bright, long-wavelength, ratioable*
*Cl\(^{-}\) sensitive (0-100 mM), pH insensitive*
*stable, non-toxic…*[1]

Sonewame, Szoka & Verkman, 2003
CALIBRATION OF A DEXTRAN CHLORIDE SENSOR

BAC-dextran-TMR

solution

cells

$\frac{F_{\text{red}}}{F_{\text{green}}}$

$[\text{Cl}^-]$ (mM)

BAC

TMR
FLUORESCENT Cl⁻ SENSING DNA-POLYAMINE POLYPLEX

dextran

DNA

polyamine

H₂N

NH₂

TMR

BAC

S

S

dextran-BAC
polyamine-TMR
ENDOCYTOSIS OF DNA POLYPEX
CHLORIDE SENSOR

**BAC-dextran-TMR-polyamidoamine / DNA**
ACIDIFICATION & Cl\(^{-}\) ACCUMULATION DEPENDS ON POLYAMINE BUFFERING

**pH**

**[Cl\(^{-}\)]** (mM)

*7.5*  
*7.0*  
*6.5*  
*6.0*  
*5.5*  
*5.0*  

*120*  
*90*  
*60*  
*30*  

**time (min)**

**0**  
**15**  
**30**  
**45**  
**60**  
**75**  

**TMR-polyamine-CF-DNA**

**BAC-dextran-TMR-polyamine-DNA**
LYSIS/PRE-LYSIS IN PEI BUT NOT POL ENDOSONOMES

Disappearance of PEI and POL over time at different pH levels.
ENDOSOME SWELLING DEPENDS ON POLYAMINE BUFFERING

endosome volume (μm³)

POL / chloroquine

PAM

POL

time (min)

POL / chloroquine

PAM

POL
‘PROTON SPONGE’ MECHANISM

Low buffering DNA polyplex

High buffering DNA polyplex
A Spectrum of Dendritic Architectures

dendrimer-star polymer hybrid

“bow-tie” polymer

dendronized hyperbranched polymer
dendronized linear polymer
Polyester Dendrimers:

Divergent synthesis with facile purification, high yields

→ Resulting dendrons are degradable, non-toxic
Dendritic Molecules for Drug Delivery

- 32 hydroxyls
- 3790 Da
- Non-toxic
- $t_{1/2} < 10$ min

- 21,000 Da
- PDI = 1.02
- Non-toxic
- $t_{1/2} = 72$ min
- Liver accumulation
- Poor solubility of drug conjugate at high loadings

Dendritic “Bow-Tie” Polymers

Poly(ethylene oxide) (PEO)
- varying molecular weights
- sterically protects payload

Dendritic Scaffold
- biodegradable polyester
- dendron generation varies degree of branching, drug loading potential

Blood Circulation of “Bow-Tie” Polymers

<table>
<thead>
<tr>
<th>Polymer &amp; MW</th>
<th>$t_{1/2\beta}$ (h)</th>
<th>% Dose in Urine (48 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[G-1]–10K (22 kDa)</td>
<td>8 ± 1</td>
<td>33 (24 h)</td>
</tr>
<tr>
<td>[G-1]–20K (44 kDa)</td>
<td>1.4 ± 0.4</td>
<td>20 (24 h)</td>
</tr>
<tr>
<td>[G-2]–5K (23 kDa)</td>
<td>11 ± 3</td>
<td>22 (24 h)</td>
</tr>
<tr>
<td>[G-2]–10K (43 kDa)</td>
<td>26 ± 6</td>
<td>18</td>
</tr>
<tr>
<td>[G-2]–20K (87 kDa)</td>
<td>25 ± 8</td>
<td>10 (24 h)</td>
</tr>
<tr>
<td>[G-3]–5K (45 kDa)</td>
<td>31 ± 2</td>
<td>2</td>
</tr>
<tr>
<td>[G-3]–10K (85 kDa)</td>
<td>40 ± 4</td>
<td>2</td>
</tr>
<tr>
<td>[G-3]–20K (160 kDa)</td>
<td>50 ± 10</td>
<td>4</td>
</tr>
</tbody>
</table>

Summary
- [G-1] polymers are rapidly cleared with most of the material in the urine and feces after 48 hours.
  Gilles et al. Mol. Pharm. 2005
Similar Mass, Different Elimination rates

<table>
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<th>[G-3]–5K (45 kDa)</th>
<th>31 ± 2</th>
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Pores Restrict Reptation of Star Polymers

Brochard-Wyat & de Gennes, 1996
Doxorubicin (Dox)

- Anthracycline antibiotic
- Dox has a well-established position in the treatment of a variety of human malignancies.
- Dox cardiotoxicity is a major clinical handicap limiting its cumulative dosage.
Drug Concentrations at a Tumor Site Following I.V. Administration

(a) Schematic representation of drug delivery to a tumor site following intravenous administration. The drug concentration in blood is denoted by $k_{elimination}$, extravasation rate $k_{extravasation}$, and tumor concentration $C^{(v)}_{free drug}$.

(b) Graph showing the concentration of free drug in tumor (% dose/g) over time (h) after intravenous injection. The half-life of release at different conditions: $t_{1/2, \text{release}}$ (h) for 500, 50, 10, and 1. The concentration peaks at different times, indicating the effectiveness of drug delivery at various conditions.
Covalent Attachment of Doxorubicin

Scheme 1

*a* hydrazide carboxylate linked doxorubicin  
*b* acyl hydrazide linked doxorubicin

$R' = \text{alkyl, aryl, not O or N}$
Synthesis Dendrimer Doxorubicin

\[ [G-3]-\text{(PEO}_{5k}\text{)}_8: 8 \text{ PEO chains (n - 110)} \]

\[ [G-4]-\text{(OH)}_{16}: 16 \text{ hydroxyls} \]

1. \( \text{O}_2\text{N} - \text{Cl} \)
   \( \text{Pyridine, CH}_2\text{Cl}_2 \)

2. \( \text{O}_2\text{N} - \text{amine} \)
   \( \text{DMAP, NEt}_3, \text{Pyridine} \)

\[ [G-3]-\text{(PEO}_{5k}\text{)}_8[G-4] \]

1. \( \text{TFA, CH}_2\text{Cl}_2 \)
2. \( \text{MeOH, 60 °C} \)

\[ [G-3]-\text{(PEO}_{5k}\text{)}_8[G-4] \]
Tumor Accumulation of “Bow Tie” Polymers

- Biodistribution of doxorubicin conjugate of G3-5K (40 kDa) performed in Balb/c mice with subcutaneous C-26 tumors
- Tumor/muscle ratio of 16 at 48 h demonstrates enhanced permeability of tumor vasculature
**Therapeutic efficacy of various control formulations in C-26 colon carcinoma in Balb/c mice.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number animals</th>
<th>Number survivors</th>
<th>Mean survival day</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>7</td>
<td>0</td>
<td>28</td>
<td>—</td>
</tr>
<tr>
<td>Acetone linked Hydrazone Bowtie 238 mg/kg polymer</td>
<td>8</td>
<td>0</td>
<td>34</td>
<td>0.051</td>
</tr>
<tr>
<td>DOX carbamate linked Bowtie 20 mg/kg DOX</td>
<td>10</td>
<td>0</td>
<td>30</td>
<td>0.019</td>
</tr>
</tbody>
</table>
C26 Tumor Progression

- PBS
- Doxorubicin 6 mg/kg
- Bow-tie 1 mg/kg
- Bow-tie 3 mg/kg
- Bow-tie 6 mg/kg
- Bow-tie 20 mg/kg
Effect of Bow-tie Dox or Doxil™ on Survival

Bow-Tie

Doxil™
Current Status

• Liposomes—mature technology
  – Excellent payload
  – Biocompatible
  – Large diameter
  – Not suitable for all drugs

• Dendritic polymers—developing technology
  – Small diameter
  – Biocompatible
  – Suitable for most drugs
  – Modest payload through chemical linkage
Nanoparticle delivery to the brain

Nanoparticles feature:

- Targeting
- Shielding
- Sensing
- Reporting

Red Blood Cell: 7 μm
Nanoparticle: 50-100 nm

Convection Enhanced Delivery

- Convective flow increases distribution to large volumes
- Direct tissue targeting

G. Huynh – Szoka group
Surface Charge Modification by Disulfide Exchange and Ligand Insertion

- Diacyl lipids
- Chol-SSR (CTL)
- PEG-lipid
- HS-R’
- Chol-SSR’
- TAT-PEG-lipid

R, positive; R’, negative or neutral
TATpeptide-targeted NLP for Brain Delivery
TAT-NLP Delivery to U87-MG Brain Tumor

No TAT ligand

With TAT ligand

(arrows: tumor border)
Intracellular drug transport

Biomolecular Adaptors for Retrograde Transport (BART)

Novel Cargo

BART peptide

Endogenous dynein

Microtubule

Rich Cohen-Szoka group
Summary

• Self-assembled systems make good drug carriers
• Improvements needed in targeting, cytoplasmic delivery and surface properties
• Sequential assembly, coupled with local delivery, can improve gene transfer to defined tissues
Acknowledgements

UCSF
- F. Szoka
- J. MacKay, Ph.D.
- E. Dy

Funded by NIH
EB3008, CA107268

UCB
- Professor Jean Frechet, Ph.D.
- Omayra Padilla, Ph.D.
- Beth Gillies, Ph.D.
- Cameron Lee
Acknowledgements

Szoka group
• J. MacKay
• X. Guo
• W. Li
• Z. Huang
• R. Eliaz
• L. Gagne
• C. Redemann
• E. Dy
• Many past members

• D. Deen, Ph.D.

• Funded by NIH
  EB3008, CA107268

The Sequus employees