20.380 workshop:
Drug Carriers: Polymers, Vesicles, Nanoparticles and systemic delivery
Images of various biological molecules removed due to copyright restrictions.
Objectives of molecular and particulate drug carriers:

- (I) Alter pharmacokinetics
- (II) Alter biodistribution
- (III) Provide drug reservoirs

Delivery via systemic and oral routes

Size limits for penetration of tissue from circulation:

Kidneys

Reticuloendothelial system
Objectives of nano- and micro-carriers: protection of cargos from premature degradation

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>APPLICATION</th>
<th>EXAMPLE HALF-LIVES IN CIRCULATION</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short peptides (5-20 amino acids)</td>
<td>Vaccine epitopes, ligands for drug targeting, peptide drugs</td>
<td>2 min., 5 min, 2 hrs</td>
<td>J. Biol. Chem. 48, 48503 (2002); J. Pharm Sci. 81, 731 (1992)</td>
</tr>
<tr>
<td>Cytokines (polypeptides typically 5-20 KDa)</td>
<td>Regulation of tissue physiology (e.g., growth factors), disease treatment (e.g., interferon-α)</td>
<td>IFN-α 3-8 hrs, interleukin-6 2.1 min, tumor necrosis factor 3 min</td>
<td>Nat Rev Drug Discov 2, 214-21 (2003)</td>
</tr>
</tbody>
</table>
Objectives of nano- and micro-carriers:
(1) protection of cargos from premature degradation

*i.v.* proteins and polymers are rapidly cleared from the blood:

Poly(N-2-hydroxypropylmethacrylamide)


Fate of injected particles in vivo

(Mebius and Kraal Nature Reviews Immunology 5, 606-616 (August 2005))

Fate of injected particles in vivo

(Braet and Wisse, Comp. Hepatology 1, 1 (2002))


(Adams and Ecksteen, Nat. rev. Immunol 6 244-251 (2006))


20.380 drug targeting workshop S09
Fate of injected particles in vivo

Carriers must avoid immune-mediated clearance to stay in circulation/traffic to target tissues


F.F. Davis (1977): showed that poly (ethylene glycol) conjugated to a protein is non-immunogenic and greatly increased protein half-lives \textit{in vivo}.


PEGylated molecules:

<table>
<thead>
<tr>
<th>Pharmacokinetic effect</th>
<th>Pharmacodynamic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interferon-α2a</strong></td>
<td></td>
</tr>
<tr>
<td>Sustained absorption</td>
<td>In vivo antiviral activity increased 12- to 135-times</td>
</tr>
<tr>
<td>Increased half-life (from 3-8 h to 65 h)</td>
<td>Anti-tumour activity increased 18-fold</td>
</tr>
<tr>
<td>Decreased volume of distribution (from 31-731L to 8-121L)</td>
<td>Improved sustained response to chronic hepatitis C</td>
</tr>
<tr>
<td>Decreased systemic clearance (from 5.6-29.2 to 0.05-0.10 L/h)</td>
<td></td>
</tr>
<tr>
<td><strong>Interleukin-6</strong></td>
<td></td>
</tr>
<tr>
<td>Increased half-life (from 2.1-208 min)</td>
<td>Thrombopoietic potency increased 500-times</td>
</tr>
<tr>
<td><strong>Tumour necrosis factor</strong></td>
<td></td>
</tr>
<tr>
<td>Increased half-life (from 3 to 45-135 min)</td>
<td>Anti-tumour potency increased 4- to 100-times</td>
</tr>
</tbody>
</table>

Table 1 | Influence of pegylation on pharmacokinetics and pharmacodynamics

Influence of pegylation on pharmacokinetics and pharmacodynamics of some therapeutic proteins, compared with corresponding native proteins (adapted from Harris, 2003).

---


Synthesis of ‘stealth’ particles

(Stolnik et al. 1995)

- Adsorbed PEG block copolymers
- Covalently grafted PEG
- Block copolymer entangled
- PEG block copolymer micelles/nanoparticles

**e.g. Pluronics:**
- PEO
- PPO

*Lecture 18 Spring 2008*

Clearance of particles from the blood

Figure showing change in dose over time from Science magazine removed due to copyright restrictions. For article, see Gref, R., et al. “Biodegradable Long-Circulating Polymeric Nanospheres.” *Science* 263, no. 5153 (1994).

(Davis et al. Nat. Rev. Drug Disc. 7 771-782 2008)

20.380 drug targeting workshop S09

TEM of nanoparticles

Release properties of diblock particles

Fig. 6. Release profiles of BSA from PLGA (○) and PEG–PLGA (●) nanoparticles.

Increased $t_{1/2}$ in blood:

Altered biodistribution:

Fig. 7. Blood clearance curves of [$^{125}$I]BSA in PLGA (○) and PEG–PLGA (●) nanoparticles.

Lecture 18 Spring 2008 (Li et al., 2001)

PASSIVE TARGETING OF TUMORS:
Enhanced permeation and retention (EPR) effect in tumors:
Enhanced permeation and retention (EPR) effect in tumors:

Applications of polymer-drug conjugates and particles as drug carriers and cellular markers

Blood-brain barrier

(Pardridge *Nat. Rev. Drug Disc.* 1 131 (2002))
Applications of polymer-drug conjugates and particles as drug carriers and cellular markers

(2) INTRACELLULAR BARRIERS: Intracellular drug delivery

Diagram showing the processes of delivery, complex formation, and intracellular barriers with labels for Cytosol and Nucleus.
OVERVIEW OF MOLECULAR/PARTICULATE DRUG CARRIERS:
STRUCTURE, SYNTHESIS, PROPERTIES

Polymer-drug conjugates

vesicles

micelles

liposomes

polymerosomes

nanoparticles

microparticles

2-10 nm

5-50 nm

50-2,000 nm

50-10,000 nm

1 nm

10 nm

100 nm

1000 nm

Images of various biological molecules removed due to copyright restrictions.
Polymer pro-drugs and markers
Polymer pro-drugs and markers

Pro-Ile-Cys(Et)-Phe-Phe-Arg-Leu

cathepsin D substrate


Figure removed due to copyright restrictions. See Figure 1 from Tung, Ching-Hsuan, et al. "Preparation of a Cathepsin D Sensitive Near-Infrared Fluorescence Probe for Imaging." Bioconjugate Chemistry 10, no. 5 (1999).
Micelle carriers

Cargo-loaded micelles via polyion association:

Figure removed due to copyright restrictions.
See Figure 3 from Kataoka, Kazunori, et al. "Spontaneous Formation of Polyion Complex Micelles with Narrow Distribution from Antisense Oligonucleotide and Cationic Block Copolymer in Physiological Saline." *Macromolecules* 29, no. 26 (1996).

Vesicle carriers

**Liposomes** – lipid bilayer vesicles formed typically using phospholipids mimicking the plasma membrane of cells

**Virosomes** – hybrids formed by fusion of liposomes with viral particles

**Polymerosomes** – synthetic vesicles formed using block copolymers as analogs of small-molecule amphiphiles
Liposome carriers

Diagram of liposome with drug entrapped in liposome interior removed due to copyright restrictions.

Fig. 2. Enzymatic conversion of Ω-AAA-DOPC to DOPE. Elastase cleaves to the C-terminal side of diacyl sequences. Cleavage generates a zwitterionic lipid from a negatively charged lipid.


Figure removed due to copyright restrictions.
Fig. 1. Putative mechanisms of enzyme activated delivery. Liposomes may be activated to become fusogenic by enzymes near the surface of the cell, enzymes displayed on the surface of the cell or enzymes in the endolysosomal compartment. Charge reversal and fusogenic delivery can occur at the plasma membrane, within an endosome or via later cleavage in the endolysosome.

Pros and cons of vesicular delivery

Advantages:

Disadvantages:
Particle surface engineering with lipids

encapsulated co-factors:

Figure removed due to copyright restrictions.
lipid segregation/self-assembly at the surface of nanoparticles

Figure removed due to copyright restrictions.
See Figures 1 and 3 from Bershteyn, Anna, et al.
"Polymer-Supported Lipid Shells, Onions, and Flowers."
**Polyplexes**

Plasmid DNA + Polycation backbone**

Hydrophilic side chains**

**Backbone components**

\[
\left( \text{NH} - \text{CH}_2 - \text{CH}_2 \right)_x \left( \text{N} - \text{CH}_2 - \text{CH}_2 - \right)_y \text{CH}_2 \text{CH}_2 \text{NH}_2
\]

PEI

\[
\left( \text{H}_2 \text{NCH}_2 \text{CH}_2 \text{CH}_2 \text{CH}_2 \text{NH} - \text{C} - \text{C} - \right)_n
\]

PLL

**Side chain components**

\[-(\text{CH}_2 - \text{CH}_2 - \text{O})_n-\] PEO

dextran

Lecture 18 Spring 2008
Nanoparticle DNA packaging

Figures removed due to copyright restrictions.
Nanoparticle DNA packaging

0.5X HBS (Hank's buffered saline) = 75 mM NaCl, 20 mM HEPES, 2.5% glucose
0.5X HBG (HEPES-buffered glucose) = 20 mM HEPES, 5% glucose

Copyright © 2001 John Wiley and Sons, Ltd. Reprinted with permission of John Wiley & Sons., Inc.

(Wightman et al., J. Gene Med. 3, 362-372 (2001))

Lecture 18 Spring 2008
Objectives of nano- and micro-carriers: targeted delivery to select tissues or cells

(Wickham *Nat. Med.* 9 135 (2003))