Lipid nanocapsules in drug delivery

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Nanomedicines

Micelles

Polymer-Drug conjugate

~6–15 nm

Nanoparticles

Liposomes

SUV

Lipid nanocapsules

MLV

Nanocrystals

Log Scale (nm)
Lipid nanocapsules (LNC)

- Lipophilic drugs in the oily core
- Hydrophilic drugs encapsulated as a microemulsion in the oily core

- PEG-hydoxystearate (Solutol®)
- Lecithin (Lipoid®)
- Triglycerides (Labrafac®)

SCIAM, University of Angers
Properties of lipid nanocapsules

Simple preparation, GRAS excipients

Adjustable size 20-150 nm

Stealth properties

Cytostatic action *in vitro* and *in vivo* to glioma cells

Capacity to inhibit P-gp

Easy scale up possible

Dispersion stability > 1 year

(Huynh et al., 2009)
Fabrication process

o/w emulsion (low T) → phase inversion zone → w/o emulsion (high T)

Low-energy method based on phase inversion induced by temperature change

dilution and/or cooling

lipid nanocapsules

(Heurtault et al., 2002)
Formulation of LNC

Feasibility domain

20-100 nm nanocapsules

(Heurtault et al. 2003)
Pilot-scale fabrication

Temperature cycles

Dilution with cold water

Batch sizes up to 50x (50 g of LNC)
Release profile from LNCs

Same loading (amiodarone), different kinetics:
polymeric nanoparticles (PLGA)
  ⇒ matrix
lipid nanocapsules
  ⇒ reservoir + membrane
GI stability of LNC and transport across intestinal epithelium

Gastric fluid: size remained stable; 12% paclitaxel released

Intestinal fluid: size stable; paclitaxel released: 6.5% in fasted state, 30% fed state

(Roger et al., Int. J. Pharm. (2009) 379, 260)

3.5-fold transport of paclitaxel across Caco-2 cells compared to Taxol® (Roger et al., J. Control. Release (2009) 140, 174)
Tumor uptake of $^{99m}$Tc-MIBI is improved by LNC and classical P-gp inhibitors *in vivo*

$^{99m}$Tc-MIBI injection s.c. of 1 million tumor cells

- Injection s.c. of 1 million tumor cells
- 9L
- Intra tumoral treatment
- 24 hour
- $^{99m}$Tc-MIBI i.v. injection
- 1 hour
- Tumor dissection & $^{99m}$Tc-MIBI uptake quantification

→ LNC inhibit MDR phenomenon *in vivo*
**Evaluation in cell culture (glioma cells)**

**9L**
- Px LNC induce 80% cell death at $2.10^{-1}$ mM (>100X)
- Taxol induces 80% cell death at 20 mM

**F98**
- Px LNC induce 80% cell death at $2.10^{-2}$ mM (>1000X)
- Taxol induces 80% cell death at 20 mM

Activation of the complement system

*CH50 test:*

1. Normal human serum (proteins of complement)
   - Non-stealth nanoparticles
   - Stealth nanoparticles

2. Sensitised erythrocytes

**Strong complement adsorption**

- Weak hemolysis
- Strong hemolysis
Activation of the complement system

- Lipid nanocapsules (LNC) and lipid nanoemulsions (LNE) activate little the complement system → good stealth properties
- Complement activation size dependent

Comparison of LNC and LNE

- CH50 unit consumption (%)
- Particle surface area (cm² / ml serum)

LNC 20 nm, LNE 20 nm, LNC 50 nm, LNE 50 nm, LNC 100 nm, LNE 100 nm, PMMA
Surface modification by post-insertion

- LNC = ”soft particles”

LNC

+ (DSPE-PEG_{2000}-COOH)

Incubation at elevated temperature
Fast dilution/cooling to 4°C

Post-inserted LNC
Surface modification by post-insertion

<table>
<thead>
<tr>
<th></th>
<th>LNC size (nm)</th>
<th>Polydispersity index</th>
<th>Zeta-potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting lipid nanocapsules</td>
<td>50</td>
<td>0.026</td>
<td>-5</td>
</tr>
<tr>
<td>DSPE-PEG₂₀₀₀⁻ COOH inserted</td>
<td>57</td>
<td>0.062</td>
<td>-42</td>
</tr>
<tr>
<td>&quot;Lipodextran&quot; inserted</td>
<td>59</td>
<td>0.045</td>
<td>-2</td>
</tr>
<tr>
<td>&quot;Lipochitosan&quot; inserted</td>
<td>56</td>
<td>0.066</td>
<td>+31</td>
</tr>
</tbody>
</table>

**Graphs:**
- **Lipodextran post-insertion**
  - Lipodextran concentration (mg/ml) vs. LNC size (nm), LNC zeta-potential (mV)
- **Lipochitosan post-insertion**
  - Lipochitosan concentration (mg/ml) vs. Size (nm), Zeta potential (mV)
Attachment of a targeting ligand

- RGD peptide binds to $\alpha_v\beta_3$ integrin on tumour cell surface
- RAFT (Regioselectively Addressable Functionalized Template): multimeric presentation of ligands

(Boturyn et al., JACS (2004) 126, 5730)

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>LNC</td>
<td>50</td>
</tr>
<tr>
<td>LNC RAFT-RGD</td>
<td>61</td>
</tr>
</tbody>
</table>
Biodistribution of targeted LNCs

Significant enhancement of brain accumulation of OX26-LNC in rats

Encapsulation of fluorescent dyes

• Commercial hydrophobic indocyanines
  – DiD, DiO, DiR, Dil, ICG

• FRET pairs
In vivo fate of LNC

LNC DiD 50 nm
• Still in blood circulation 24 h after injection
**In vivo fate of LNC**

LNC DiD 50nm, 24h after injection

Fluorescence intensity (a.u.)

- **Solutol**
- **Lipodextran**
- **Lipochistosan**

"Normal" LNCs (Solutol) or coated with dextran or chitosan
Project partners and funding

- **Inserm U646, Angers**
  - Samuli HIRSJÄRVI, Emmanuel GARCION Catherine PASSIRANI, Olivier THOMAS, Jean-Pierre BENOIT

- **DTBS CEA-LETI, Grenoble**
  - Julien GRAVIER, Isabelle TEXIER

- **Laboratoire Colloïdes et Matériaux Divisés, ESPCI, Paris**
  - Yan QIAO, Audrey ROYERE, Jérôme BIBETTE

- **Inserm U823, Grenoble**
  - Sandrine DUPORT, Jean-Luc COLL

- **Personal funding**
  - Academy of Finland, Alfred Kordelin Foundation, L'Association Franco-Finlandaise pour la Recherche Scientifique et Technique