Comparative in-vitro characterization of liquid liposomal formulations

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From Gregoriadis, 1979
Issues covered

Liposomes……
– introduction/positioning
– components
– structure
– morphology
– manufacturing, stability
– quality control, FDA, EMA

9/24/2012

Approved Liposome-based Drug Products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Year Approved</th>
<th>API</th>
<th>Sparingly Soluble</th>
<th>Revenue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visudyne®</td>
<td>2000</td>
<td>Verteporfin</td>
<td>Yes</td>
<td>90 M</td>
</tr>
<tr>
<td>DOXIL/Caelyx®</td>
<td>1995</td>
<td>Doxorubicin</td>
<td>No</td>
<td>550 M $*</td>
</tr>
<tr>
<td>AmBisome®</td>
<td>1990</td>
<td>Amphotericin B</td>
<td>Yes</td>
<td>400 M $*</td>
</tr>
<tr>
<td>ABELCET®</td>
<td>1995</td>
<td>Amphotericin B</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Definity®</td>
<td>2001</td>
<td>Octafluoropropane</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Myocet®</td>
<td>2001</td>
<td>Doxorubicin</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>DepoCyte®</td>
<td>2002</td>
<td>Cytarabine</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>DepoDur®</td>
<td>2004</td>
<td>Morphine</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Daunoxome®</td>
<td>1996</td>
<td>Daunorubicin</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Octocog alfa®</td>
<td>2009</td>
<td>Factor VIII</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

* Thomson Pharma
** not exhaustive

www.northernlipids.com
What is in the pipeline?

Thomson-Pharma, 2010

Liposomes: which actives?

Hydrophilic molecules
- S-fluorouracil
- cisplatin
- cyclosporin
- paclitaxel
- 5'-fluorouracil
- cisplatin
- daunorubicin
- doxorubicin
- dibucaine
- ibuprofen
- paclitsal
- cyclosporin
- lidocaine

Amphiphilic molecules
- Depending on the partition coefficient
- Covers a wide range of molecules
- bupivacaine
- ferulic acid
- daunorubicin

Weak bases and weak acids
- Covers a wide range of molecules
- doxorubicin
- cisplatin
- DNA
- RNA

Nucleic acid based-drugs
(Phospho)lipids

\[ \text{PC} \]
\[ \text{PE} \]
\[ \text{PG} \]
\[ \text{DOTAP} \]

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\[ \text{HO-C}_n\text{H}_{2n+1} \text{O-C}_n\text{H}_{2n+1} \text{O-R} \quad R=\text{fatty alcohols } C_{12}-C_{18} \quad n=2-6 \]

Polyoxyethylene alkylether

\[ \text{HO-C}_n\text{H}_{2n+1} \text{O-C}_n\text{H}_{2n+1} \text{O-CO-R} \quad R=\text{fatty acids (saturated/unsaturated) } C_{12}-C_{18} \quad n=\text{ca.2-6} \]

Polyoxyethylene alkylester

\[ \{ \text{Hydrophilic part} \} \]

\[ \{ \text{Alkylchain} \} \]

Saccharosediester

Non-ionic, ‘synthetic’ surfactants
Factors controlling the fate of liposomes *in vivo* after intravenous administration:

- size of the liposomes (0.03 - 20 µm)
- type (morphology) (unilamellar, multilamellar, multivesicular)
- charge of the bilayer (negative, neutral, positive)
- rigidity of the bilayer (gel/fluid state)
- route of administration
Evolution in Liposome Science

- 1\textsuperscript{st} generation
  - conventional liposomes
- 2\textsuperscript{nd} generation
  - immunoliposomes
  - long circulating liposomes
  - cationic liposomes
Some pharmaceutical aspects:
What are potential problems encountered in the development process of liposomes?

- poor quality of the raw material, the phospholipids
- poor characterization of the physico-chemical properties of the liposomes
- ‘pay load’ is too low
- shelf life is too short
- scaling up problems occur
- absence of (any) data on safety of these carrier systems on chronic use
- ........

Apparently, the above issues have not stopped the successful launching of drug-liposome products on the market....

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Sterility problems

FDA Update on Doxil and Methotrexate Shortages

Today, FDA announces actions taken to bolster the supply of Doxil® (Janssen Research & Development, LLC) and preservative-free formulations of methotrexate.

Liposomal doxorubicin

In response to the critical shortage of Doxil® (doxorubicin hydrochloride liposome injection), effective immediately the U.S. Food and Drug Administration is exercising its enforcement discretion for the temporary importation and distribution of Sun Pharma Global's Lipodox™ (doxorubicin hydrochloride liposome injection) in the United States by Sun Pharma Global PTE and its authorized distributor, Cawan Pharmaceutical - I Laboratories, Ltd.

Administration is exercising its enforcement discretion for the temporary importation and distribution of Sun Pharma Global's Lipodox™ (doxorubicin hydrochloride liposome injection) in the United States by Sun Pharma Global.
(Phospho)lipids

PC

PE

PG

DOTAP
Liposome classification:

THE liposome does not exist!!!

- **A) Based on structural parameters**
  - MLV: Multilamellar large vesicles - > 0.5 µm
  - OLV: Oligolamellar vesicles - 0.1 - 1 µm
  - UV: Unilamellar vesicles (all size range)
  - SUV: Small unilamellar vesicles - 20-100 nm
  - MUV: Medium sized unilamellar vesicles
  - LUV: Large unilamellar vesicles - >100 nm
  - GUV: Giant unilamellar vesicles (vesicles with diameters > 1 µm)
  - MVV: Multivesicular vesicles (usually large > 1µm)

- **B) Based on method of liposome preparation**
  - REV: Single or oligolamellar vesicles made by reverse-phase evaporation method
  - MLV-REV: Multilamellar vesicles made by the reverse phase evaporation method
  - SPLV: Stable plurilamellar vesicles
  - FATMLV: Frozen and thawed MLV
  - VET: Vesicles prepared by extrusion methods
  - DRV: Dehydration-rehydration vesicles

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**Type of Vesicle Geometry**

- SUV
- LUV
- MLV (classical)
- MVV
- OLV
- MLV (MLV-REV, SPLV, FATMLV)

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Homogenizers and Extruders

- Avestin (Canada)
- APV (German)
- Microfluidics (US)
Loading of liposomes with drugs

**Passive** loading:
- drug dissolved in organic or aqueous medium during liposome formation
  - loading efficiency is 5 - 100%, depending on hydrophobicity, vesicle size and preparation method

**Active** loading (after sizing; 11 patented methods):
- up to 100% loading efficiency for drugs with a pH dependent partition-coefficient

Active Loading of Doxorubicin by Ammonium Sulfate Gradient Method

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Structure of Doxil®

- **Doxorubicin**
- **Lipid Membrane (Phospholipid + Cholesterol)**
- **Polyethylene Glycol**

**Doxil Process**

1. **Hydration**
   - Lipids
   - Ethanol
   - Ammonium Buffet
   - Unsize Liposome Formation

2. **Down-Sizing**
   - Extrude sequentially through capillary pore filters of decreasing pore diameter

3. **Buffer Exchange Ethanol Removal**
   - Sucrose
   - Flow Dilutes
   - Filtrate

4. **Drug Loading**
   - Add Doxorubicin HCl

5. **Dilution**
   - Measure Drug Conc/QS to Label Strength

6. **Sterilization and Fill**
   - Sterile filter through 0.22 μ filter
   - Aseptic Fill

**From Frank Martin**

- From 9/24/2012
Different bilayers

Same physicochemical characteristics

Does it make a difference in vivo?

Pharmacokinetics, efficacy and toxicity of different pegylated liposomal doxorubicin formulations in preclinical models: is a conventional bioequivalence approach sufficient to ensure therapeutic equivalence of pegylated liposomal doxorubicin products?

Table 1 The composition of liposomal formulations and its characteristics

<table>
<thead>
<tr>
<th>Formulation #</th>
<th>Potency (mg/mL)</th>
<th>% encapsulation</th>
<th>Particle Size (nm)</th>
<th>pH</th>
<th>Phosphorus (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation 1 (Doxil-control)</td>
<td>2.09</td>
<td>97</td>
<td>142</td>
<td>6.5</td>
<td>0.42</td>
</tr>
<tr>
<td>Lipid composition: DSPC:CHOL:mPEG-DSPE (56:4:38.3:5.3); Internal buffer: 250 mM ammonium sulfate; Total lipid: 21.9 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formulation 2</td>
<td>1.97</td>
<td>97</td>
<td>118</td>
<td>6.6</td>
<td>0.47</td>
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<tr>
<td>Lipid composition: DSPC:CHOL:mPEG-DSPE (56:4:38.3:5.3); Internal buffer: ESAS 100 mg/mL; Total lipid: 24.5 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formulation 3</td>
<td>2.04</td>
<td>97</td>
<td>123</td>
<td>6.5</td>
<td>0.40</td>
</tr>
<tr>
<td>Lipid composition: DSPC:CHOL:mPEG-DSPE (56:4:38.3:5.3); Internal buffer: Sucrose octasulfate; Total lipid: 20.9 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formulation 4</td>
<td>1.96</td>
<td>94</td>
<td>125</td>
<td>6.4</td>
<td>0.35</td>
</tr>
<tr>
<td>Lipid composition: DSPC:CHOL:mPEG-DSPE (56:4:38.3:5.3); Internal buffer: 250 mM ammonium sulfate; Total lipid: 18.9 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. The composition of liposomal formulations and its characteristics

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<tr>
<th>Formulation #</th>
<th>Lipid composition</th>
<th>Internal buffer: 250 mM ammonium sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Doxil control)</td>
<td>DSPE/CHOL: mPEG-DSI</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>DMSO/CHOL: mPEG-DSI</td>
<td>DSAS 100 mg/ml; Total 1</td>
</tr>
<tr>
<td>3</td>
<td>DSPE/CHOL: mPEG-DSI</td>
<td>Sucrose octasulfate; Total 1</td>
</tr>
<tr>
<td>4</td>
<td>DSPE/CHOL: mPEG-DSI</td>
<td></td>
</tr>
</tbody>
</table>

Pharmacokinetics, efficacy and toxicity of different liposomal doxorubicin formulations in preclinical models need to be carefully evaluated to ensure therapeutic equivalence of pegylated liposomal products.

Kuo N, V A Maxwell, Steve Wang, Susan Stefan, Charles Wang, Ming Yu, Tony Huang, Alfred P. Tung, Michael P. Kelley, Anthony Angiulli, Shi-Chung Fang

Fig. 2. Efficacy (changes in % mean tumor volumes) of different pegylated liposomal products in established human breast (MDA-MB-231) tumor xenografts after i.v. administration (once weekly for 3 weeks) to mice at 2 mg/kg dose. (Each data point is mean ± SE of n = 10 observations.)
Stability issues re aqueous liposome dispersions (shelf life preferably > 2 years)

- chemical degradation
  - hydrolysis reactions of lipids
  - oxidation of lipids

- physical instability
  - aggregation
  - fusion
  - leakage of drug

Solutions to potential stability problems: (freeze) drying, careful selection of (phospho)lipids, filling conditions, ....

(Phospho)lipids

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GMP compliant production

+ Aseptic processing vs. end sterilisation

+ Product specific validation of filters on microbial retentivity

+ Quality/origin of lipids (natural vs. synthetic)

+ Production process: type of liposomes governs type of process
To assure the quality of liposomal formulations a number of evaluation tests are available:

<table>
<thead>
<tr>
<th>Assay/Methodology/Analytical Target</th>
<th>Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>pH meter</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>Osmometer</td>
</tr>
<tr>
<td>Phospholipid concentration</td>
<td>Lipid phosphorus content/HPLC</td>
</tr>
<tr>
<td>Phospholipid composition</td>
<td>TLC, HPLC</td>
</tr>
<tr>
<td>Cholesterol concentration</td>
<td>Cholesterol oxidase assay, HPLC</td>
</tr>
<tr>
<td>Drug concentration</td>
<td></td>
</tr>
</tbody>
</table>

**Chemical stability**

<table>
<thead>
<tr>
<th>pH</th>
<th>pH meter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipid peroxidation</td>
<td>conjugated dienes, lipid peroxides</td>
</tr>
<tr>
<td>Phospholipid hydrolysis</td>
<td>FA composition (GLC)</td>
</tr>
<tr>
<td>Cholesterol autooxidation</td>
<td>HPLC, TLC</td>
</tr>
<tr>
<td>Antioxidant degradation</td>
<td>HPLC, TLC</td>
</tr>
</tbody>
</table>

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Based on Barenholz an Crommelin, 1994)

To assure the quality of liposomal formulations a number of evaluation tests are available:

- **Physical stability**
  - Vesicle size distribution
    - submicron range
    - micron range Coulter Counter, light microscopy, laser diffraction, GEC
  - Electrical surface potential, surface pH/zeta-potential measurements, pH sensitive probes
  - Numbers of bilayers SAXS, NMR
  - Percentage of free drug GEC, IEC, protamine precipitation
  - Dilution-dependent drug release retention loss on dilution
  - Relevant body fluid induced leakage GEC, IEC, protamine precipitation

- **Biological characterization**
  - Sterility aerobic and anaerobic cultures
  - Pyrogenicity rabbit or LAL test
  - Animal toxicity monitor survival, histology, pathology

(Based on Barenholz an Crommelin, 1994)

SAXS = small angle X-ray scattering, DLS = dynamic light scattering, GEC = gel exclusion chromatography, IEC = ion exchange chromatography, LAL = Limulus Amoebeocyte Lysate, NMR = nuclear magnetic resonance, SAXS = small angle X-ray scattering, TLC = thin layer chromatography.
Particle size measurement

- Electron microscope:
  - negative staining
  - freeze fracture
  - cryo-TEM

- Dynamic light scattering
  - Typical instrumentation is manufactured by e.g. Malvern Instruments Ltd. (UK), Beckman Coulter, Inc. (USA), Nicomp (USA) and ALV-GmbH, Germany)

Cryo-TEM
Electron microscope pictures of liposome dispersion sequentially extruded through membranes: from 0.6 micrometer down to 0.2 micrometer.

Bar 0.5 micrometer

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**In Vitro release**

- Media: 50 ml HEPES buffer, 10 mM, pH 7.4
- Membrane: Spectrapor Disposdialyzer 50 kDa MWCO

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**Dialysis sac**

- Media: 125 ml HEPES buffer, 10 mM, pH 7.4
- Membrane: Spectrapor Disposdialyzer 50 kDa MWCO

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N. Chidambaram and D.J. Burgess. AAPSPharmSci., (1999), August 31, 1999; 1(3)
Guidance for Industry

Liposome Drug Products

Submission of Chemistry, Manufacturing, and Controls, Human Pharmacokinetics and Bioavailability and Labeling Information

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the Federal Register of the notice announcing the availability of the draft guidance. Submit comments to Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857. All comments should be identified with the docket number listed in the notice of availability that publishes in the Federal Register. For questions regarding this draft document contact XXX.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
XXX, 2001

Mei-Ling Chen

9/24/2012

FDA issues

• Bioavailability/Bioequivalence
  – release of active moiety from liposomes
  – availability at site of action

• CMC issues
  – characterization of drug product
  – physicochemical characteristics
    • size, lamellarity, free/associated

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Shaw/Kumi
Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product

Draft

Conclusions

- **The** liposome does not exist
- Liposomes made it to the market because:
  - Basic know how on their behavior in vivo was collected over years
  - Pharmaceutical challenges were adequately met