Carrier mediated targeted delivery of drugs to endothelial cells in disease.

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Laboratory for Endothelial Biomedicine & Vascular Drug Targeting research

1st International Conference on Sustainable Pharmacy

24-25 April 2008

Osnabrück, Germany
Drugtargeting

Without targeting, whole body distribution of the drug

With targeting selective delivery of the drug at the site (organ/cell) of the disease

• Higher concentration of the drug at the site of disease

• Less unwanted side effects
Drug delivery: toward versatile formulation technology for broad application to existing drugs/NME

“The synthesis of the medicine is only one part of the drug. Without delivery you just won’t have a successful treatment”
Bob Langer / MIT, FDA - 2003
<table>
<thead>
<tr>
<th>Drug or therapeutic agent (trade name), manufacturer(s)</th>
<th>Indication</th>
<th>Year of approval</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomal amphotericin B (AmBisome), Gilead, Fujisawa</td>
<td>Fungal infections</td>
<td>1990 (Europe), 1997</td>
<td>(44)</td>
</tr>
<tr>
<td>PEG-adenosine deaminase (Adagen), Enzon</td>
<td>Leishmaniasis</td>
<td>2000</td>
<td></td>
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<td></td>
<td>Severe combined immunodeficiency disease</td>
<td>1990</td>
<td>(45)</td>
</tr>
<tr>
<td>Styrene maleic acid and neocarzinostatin copolymer in Ethiodol (SMANCS/Lipiodol, Zinostatin stilbamamer), Yamanouchi</td>
<td>Hepatocellular carcinoma</td>
<td>1993 (Japan)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1996 (Japan)</td>
<td>(46, 47)</td>
</tr>
<tr>
<td>Stealth (PEG-stabilized) liposomal doxorubicin (Doxil/Caelyx), ALZA, Schering Plough</td>
<td>Kaposi’s sarcoma</td>
<td>1995</td>
<td>(10, 48)</td>
</tr>
<tr>
<td></td>
<td>Refractory ovarian cancer</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Refractory breast cancer</td>
<td>2003 (Europe, Canada)</td>
<td></td>
</tr>
<tr>
<td>Liposomal cytosine arabinoside (DepoCyt), SkyePharma</td>
<td>Lymphomatous meningitis</td>
<td>1999</td>
<td>(25, 49)</td>
</tr>
<tr>
<td></td>
<td>Neoplastic meningitis</td>
<td>Phase IV</td>
<td></td>
</tr>
<tr>
<td>Denileukin diftitox or interleukin 2–diptheria toxin fusion protein (ONTAK), Seragen</td>
<td>Cutaneous T-cell lymphoma</td>
<td>1999</td>
<td>(50)</td>
</tr>
<tr>
<td>Liposomal doxorubicin (Myocet), Elan</td>
<td>Metastatic breast cancer in combination with cyclophosphamide</td>
<td>2000 (Europe)</td>
<td>(51)</td>
</tr>
<tr>
<td>Gemtuzumab ozogamicin or anti-CD33–linked calicheamicin (Mylotarg), Wyeth-Ayerst</td>
<td>CD33(^\text{+}) relapsed acute myeloid leukemia</td>
<td>2000</td>
<td>(52)</td>
</tr>
<tr>
<td>Liposomal verteporfin (Visudyne), QLT, Novartis</td>
<td>Wet macular degeneration in conjunction with laser treatment</td>
<td>2000</td>
<td>(30)</td>
</tr>
<tr>
<td>PEG–interferon (\alpha-2b) (PEG-Interon), Enzon, Schering–Plough</td>
<td>Hepatitis C</td>
<td>2001</td>
<td>(53)</td>
</tr>
<tr>
<td>PEG–granulocyte colony stimulating factor or pegfilgrastim, (Neulasta), Amgen</td>
<td>Reduction of febrile neutropenia associated with chemotherapy</td>
<td>2002</td>
<td>(54)</td>
</tr>
<tr>
<td>(^{90})Y–ibritumomab tiuxetan or (^{90})Y anti-CD20 (Zevalin), IDEC</td>
<td>Relapsed or refractory non-Hodgkin’s lymphoma</td>
<td>2002</td>
<td>(55)</td>
</tr>
<tr>
<td>(^{131})I–tositumomab (anti-CD20) (Bexxar), Corixa, GlaxoSmithKline</td>
<td>CD20(^\text{+}) relapsed non-Hodgkin’s lymphoma</td>
<td>2003</td>
<td>(56)</td>
</tr>
</tbody>
</table>

Allen & Cullis (2004), Science 303, 1818
Carriers for (vascular) drug targeting

**Peptides**  
(doxorubicin, apoptosis inducing ligands)

**Growth factors**  
(toxins)

**Antibodies**  
(toxins, toxic drugs, radioisotopes)

**Viruses**  
(therapeutic genes)

**Polymer based nanoparticles**  
(therapeutic genes, toxic drugs)

**Liposomes**  
(toxic drugs)

**Immune effector cells**  
(cytolytic activity)

**Drug carrier requirements**

- Stability in the blood circulation
- Efficient homing (accessibility of target organ/cells)
- Dissociation carrier/drug (intracellular release)
- Intracellular homing

Innovation focus drug delivery:
Targeted drug delivery systems based on materials that are biocompatible, can contain pharmacologically effective NCEs, and possess intracellular release modalities

Laboratory for Endothelial Biomedicine and Vascular Drug Targeting research
Dept. Pathology & Medical Biology, Medical Biology section, UMCG

Microvascular endothelial cells as target:
• control disease activity
• easily accessible for nanomedicines
• new technologies enable in vivo studies on pharmacological efficacy of targeted drug delivery systems → animal and human studies
glomeruli (Glom)
venular EC (V)
arteriolar EC (A)
qRT-PCR

Mouse models

in vitro cell systems
and advanced readout systems

Advanced in vivo pharmacology
readout systems

UMCG EC facility

CD31
VE cadherin
control acute glomerulonephritis
whole kidney
arteriolar EC
glomeruli
venular EC

Advanced in vivo pharmacology
readout systems

UMCG EC facility

qRT-PCR

control
acute glomerulonephritis
Endothelial cells: Targets for selective liposome mediated drug delivery

• Involvement in patho-physiological processes
• Accessibility for substances transported by the blood
• Endothelial cell heterogeneity, allowing for organ specific and/or disease specific drug delivery strategies

E.g. Endothelial cells express or over-express a variety of adhesion molecules during inflammation
Endothelial cells

- Covering the inside of the blood vessels
- Surface 350 m²; $1.10^{13}$ cells

Functions:
- Transport of substances from the blood to underlying tissue
- Blood coagulation
- Inflammation
- Angiogenesis
Endothelial cell activation: a key step in inflammatory response

TNFα, IL-1

IκB degradation

Adhesion molecules: Targets for selective liposome mediated drug delivery in inflammation

Chemokines  Cytokines  E-selectin  ICAM-1  VCAM-1
Targeting of immunoliposomes in glomerulonephritis
Drug targeting to inflamed endothelium via E-selectin: anti-E-selectin immunoliposomes
Targeting of immunoliposomes in glomerulonephritis

Day – 6.5
Development
Immuneresponse

Sacrifice animals
Harvest kidneys

(b) + MES1-dexa-liposome (0.4 μmol lipid)
(c) + IgG-dexa control liposome (0.4 μmol lipid)
(d) + free dexa

Kidney uptake of AbEsel - immunoliposomes is four fold higher compared to non-targeted immunoliposomes 24 h after i.v. injection

**AbEsel** liposome

**IgG** liposome

Tissue Uptake

<table>
<thead>
<tr>
<th>Tissue</th>
<th>nmol lipid per g organ</th>
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</thead>
<tbody>
<tr>
<td>liver</td>
<td>250±12</td>
</tr>
<tr>
<td>spleen</td>
<td>150±12</td>
</tr>
<tr>
<td>kidney</td>
<td>10±12</td>
</tr>
<tr>
<td>heart</td>
<td>5±1</td>
</tr>
<tr>
<td>lung</td>
<td>5±1</td>
</tr>
</tbody>
</table>

**Targeting index**

- liver: 0.4
- spleen: 0.6
- kidney: 3.6
- heart: 1.5
- lung: 0.6

AbEsel-immunoliposomes containing dexamethasone down-regulate adhesion molecule expression only in glomerular endothelium

LDM

Glomeruli

VCAM-1 expression

Aterioles and venules
AbEsel-immunoliposomes containing dexamethasone reduce renal injury in glomerulonephritis

* p=0.03  * p=0.03

* p=0.04  p=0.09

Ásgeirsdóttir
Kamps et al,
Mol. Pharmacol.
2007, 72: 121
Targeted dexamethasone was devoid of systemic side effects

- Upper body obesity with thin arms and legs
- Buffalo Hump
- Red, Round Face
- High Blood Sugar
- High Blood Pressure
- Vertigo
- Blurry Vision
- Acne
- Female Balding
- Water Retention
- Menstrual Irregularities
- Thin Skin and Bruising
- Purple Striae
- Poor Wound Healing
- Hirsutism
- Severe Depression
- Cognitive Difficulties
- Emotional Instability
- Sleep Disorders
- Fatigue
Conclusion

Targeted delivery of dexamethasone using anti-E-selectin IL leads to local effects on gene expression, diminished side effects and reduced progression of glomerulonephritis.
Sustainable Pharmacy

Drug targeting: Reduction of administration of pharmaceutically active compounds?

More/new drugs?

Reduction of environmental emissions?
60% of the injected dose of Aco-HSA liposomes disappear fast from the blood and are taken up by liver endothelial cells.

*Bartsch et al. Mol. Pharmacol. 2005*  
*Kamps et al. PNAS 1997*
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