Basic considerations on the use of particles and polymers in molecular imaging

in “Chemistry in Contrast Media”

Hisataka Kobayashi, MD, PhD,
Molecular Imaging Program
NCI/NIH

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Learning objects

- Non-targeted particles and polymers
- “Really” basic considerations
- Inspiration from nature’s own particles
- Clearance pathways in relationship to size and surface properties (e.g., PEGylation)
- Size aspects in relation to dimensions of biological entities
- Surface-to-volume
- Interactions with blood components
- Immunogenic effects

Case by case for each polymer or nano-particle!!

Contents

- Basic consideration on nano-materials
  - “slow but powerful”, unique properties…
  - Bio-degradable?
- Pharmacokinetics (PK) of nano-sized agents
  - Size
  - Beyond size
    • charge, hydrophilicity, surface-coating, flexibility
    • Nano-toxicology
- Interaction with protein
- Immunogenicity

Small vs Large

Small molecules
Polymer and nano-materials

Tracks: Slow but powerful
Bikes: Fast but less powerful
Can be faster, safer, or more powerful???

Unique signaling

Nanomedicine for imaging

Imaging probe design

Vehicle/Platform
Targeting ligand
Target molecule
Nano-toxicology

1. Delivery/kinetics
2. Multi-valency
   polymers/nano-particles
Organs specific imaging
Nano-medicine

Special signaling
Multi-color

Quantum dots can emit multiple color of light with single excitation
Upconverting Nano-crystals (UCNC)
can emit shorter wavelength of light than excitation light.
can realize imaging without background auto-fluorescence.

Biodegradable?
Non-biodegradable
• Generally a covalently-bonded single molecule without enzyme for catabolism.
• PK: an injected molecule simply behaves depending on its physical and chemical characteristics.

Biodegradable
• A molecule with cleavable bonds, or a self-assembled crystal or particle consisting of multiple molecules or ions
• PK: complicated! because all intermediate and final catabolites can behave differently in the body.

To explain the basic strategy, I only discuss the behavior of non-degradable molecules/reagents.

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We learned PK of nano-sized agents a lot from radio-labeled antibody studies

Pharmacokinetics of nano-sized agents
• Size
• Beyond size
  – Charge
  – Hydrophilicity
  – Hard/soft – shape/flexibility
  – Binding or association with serum proteins
Dendrimer-based MRI contrast agents

We used this series of molecules as cores to synthesize the Gd-based nano-size contrast agents with various sizes but identical chemical properties.

Dynamic MRI

Liver + kidney excretion

Kidney excretion

Increased blood intensity

Liver

Biodistribution differences of nano-sized molecules

The body can well recognize the differences of nano-sized molecules.
Renal selection of nano-sized molecules

Size of molecules; major league

- small: 3 nm (G2)
- 6 nm (G4)
- 8 nm (G6A)
- 13 nm (G8)

Blood pool

- Glomerular filtration

Urine

Renal selection of nano-sized molecules

PAMAM-G7 (10 nm)

PAMAM-G9 (13 nm)

PAMAM-G3 (5 nm)

Gd-DTPA (<1 nm)

There is a cut-off nano-size which can regulate the renal excretion.

Nano-sized particles’ behavior in kidney

Renal selection of nano-sized molecules

Renal excretion

Safer in toxicity

Longer circulation

High input function (EPR effect)

Size of nano-materials for imaging

Pharmacokinetics in nano-sized P&P

In terms of PK of nano-molecules, three major players are...

1. Vascular wall is a player for molecules with <3 nm in diameter by the almost free extravasation of molecules with the glomerulus.
   - Tumor vessels are leakier than normal capillary
2. Kidney is a player for molecules with <6 nm in diameter by the filtration of molecules with the glomerulus.
   - Rapid clearance. Safer profile for toxicology
3. Liver is a player for molecules with >20 nm in diameter by the recognition of molecules with the RES.

Leakage from the vessels

capillary

large vessels

- <3 nm in diameter
- >3 nm in diameter

Tumor vessels are leakier than normal capillary


(Choi, HS., Nature Biotech 2006)

(Longmire M, Kobayashi H., Nanomedicine 2008)
Beyond size

Charge

PK change of Fab (~6nm) by charge

PamAM-G4

PPI/DAB-G4

1 min after injection

Bright liver with PPI

9 min post-injection

PK change of Fab (~6nm) by charge

Beyond size

Hydrophilicity
(Stealthy from RES)

Different interior PAMAM to PPI change PK

Beyond size

Hard/soft – Shape/Flexibility
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**PK of nano-materials beyond size**

- **(hard/soft interior)**
  - Lysine core/interior: G3
    - 14.5 nm/~50 kD
    - 127 terminals
  - Ammonia core PAMAM-G6
    - 10.5 nm/~150 kD
    - 192 terminals
  - EDA core PAMAM-G6
    - 15 nm/~200 kD
    - 256 terminals

- **(shape)**
  - PAMAM-G4 PEG2k x60
    - 18 nm~140 kD
    - 64 terminals
  - PAMAM-G4 PEG2k x2
    - 18 nm~100 kD
    - 62 terminals

**Binding or association to protein**

- **Strong binding**
  - Behave like a single larger molecule
  - Longer clearance from the circulation

- **Weak association**
  - Partially behave like a single larger molecule in short term
  - Clearance does not change much

**Immunogenicity**

- Immunogenicity of nano-sized agents is Yes or No answer, yet is really case-by-case and hard to be predicted.
- Lowering immunogenicity: surface coating
  - Well hydrophilic (i.e. PEG)
  - Neutral or a little anionic surface charge

- Less interaction with immune cells
- Less opsonization
Hydrophilic (PEG) surface coating

Plasma half life = 12 h
Plasma half life = 0.3 h

Hydrophilic coating induces stealthy of nano-sized molecules/ particles from RES

(Kojima C, Kobayashi H., Int J Pharm. 2010 and many others)

Summary

• Nano-materials with relatively small size can be excreted through kidneys into urine, resulted in preferable profile for the nano-toxicology.
• The in vivo delivery and BioD of nanomaterials can be controlled by simply changing the physical and chemical characteristics (size, shape, charge, flexibility, hydrophilicity, surface coating, etc.).
• Long circulation due to stealthy from RES is important for tumor delivery based on EPR effects
• Signal obtained from nano-materials can be unique for depicting new organs or targets, which currently cannot be visualized.

Lymph node imaging

Mechanism

Post USPIO Histology

Mets
Mets+


Dynamic MR lymphangiography of a pig

(Gd-dendrimer agent: 1 μmolGd/kg)

Optical lymphatic flow imaging using ICG

ICG is an FDA-approved fluorophore. ICG can bind to serum protein and behave like an macromolecule in vivo.

(Trojan, Frangioni, Ann Surg Oncol 2009)

Take home messages

1. Nano-material (polymers or particles) can have nearly infinite possibilities for developing new imaging agents.
2. Precise control of size, shape, charge, hydrophilicity, and flexibility allows us to optimize the target-delivery and pharmacokinetics of imaging agents in the body that can improve imaging and lower nano-toxicity.
3. Unique signaling characteristics allows us to perform a variety of multiplexed imaging, which can extract more comprehensive information from the living body than conventional imaging.