Gadolinium Specification via ICP-MS, GPC & Laserablation

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Conflicts of Interest

Bayer employee.
Brain sections:
- Cerebrum
- Cerebellum
- Pons

**Brain Tissue Fractionation and Separation**

- **Tissue homogenate**
  - **Sonication**
  - **Centrifugation**
  - **Involute (pellet)**
  - **Soluble (supernatant)**

**ICP-MS**
- Allows quantification of total Gd concentration, but does not discriminate between chemical species.

**Potential insoluble components** (not investigated here):
- Inorganic Gd precipitates (e.g. phosphate)
- Gd bound to insoluble tissue components

**Separation by GPC**:
- Gd in macromolecules
- Gd-complex (small molecule)

**Gel Permeation Chromatography, GPC**
- A chromatographic procedure to separate high and low molecular weight components.
- ICP-MS was used to identify Gd containing components (detected as chromatographically separate peaks).

A control study was performed in a similar way with blank tissue, spiked with low amounts of all GBCAs to assess the recovery of Gd in each step.
Significantly higher gadolinium concentrations in the cerebellum for all linear GBCAs: highest concentrations for Omniscan®, no difference between Magnevist® & Multihance® - no complete elimination of Gd over time.

Macrocyclic GBCA showed decreasing gadolinium concentrations in the cerebellum between 5 weeks, 1 year and between 26 weeks and 1 year - Long-lasting, slow elimination process of GBCA from cerebellum.
Differences linear vs. macrocyclic GdCAs: Chromatography of aqueous Cerebellum Homogenates divided Gd into GBCA, insoluble Gd and macromolecular Gd

Formation of Gd-macromolecules with multipurpose linear GdCAs (Magnevist®, MultiHance® & Omniscan®) results in a visual signal increase in the brain

Not visible with macrocyclic agents

Quantification and Assessment of the Chemical Form of Residual Gadolinium in the Brain After Repeated Administration of Gadolinium-Based Contrast Agents: Comparative Study in Rats. Frenzel T, Apte C, Jost G, Schöckel L, Lohrein J, Pietzsch H.
Long term study of gadolinium presence
Gd-distribution (Laser Ablation – ICP-MS)

Methodology: 1.) MRI                       2.) Microscopy     3.) Laserablation coupled plasma mass spectrometry (ICP-MS)

Parameters:
Histological slice: 30 µm
Laser energy: 29%
Laser spot size: 60 µm Ø
Scan speed: 70 µm/s
No of lines: ca. 100, measurements per line: ca. 1000
pixel size: ca. 90 x 90 µm

• Genuine speciation by molecular mass (mass/charge)
• Speciation maps possible
• Sample preparation involves chemical treatment, variable volatilization of species in sample & in mass spec
• Expensive equipment, experts, and is time consuming
What we could know using these methods:

- The nature of the Gd-binding macromolecules (protein, carbohydrate, pigments etc.)
- Is the Gd$^{3+}$ ion binding to the macromolecule (most likely) or the intact GBCA (less likely, since they all do not bind to plasma proteins) – isolate protein, separate Gd species, and identify.
- The insoluble fraction is probably very inhomogeneous and may contain diverse species:
  - Inorganic Gd (as phosphate or hydroxide) which can be detected as dense Gd clusters in EM-EDX, co-localized with phosphorus
  - Gd$^{3+}$ ion or GBCA entrapped in membrane vesicles or bound to insoluble tissue components, such as lipophilic macromolecules (neuromelanin or other pigments)
  - This fraction will be very difficult to analyze and characterize but contains about 50% of the Gd from linear GBCAs
- Comparison of the Gd-level found in brain with other environmental problematic elements, Cd, Al or Pb in brain.
- Speciation and trafficking of GBCA and metabolites over time / tissues
Thank you!

Bye-Bye
What we know about speciation:

- Gd from linear agents has been found in three different groups of chemical species:
  - A soluble fraction of low molecular weight (intact GBCA) which is also excreted from brain slowly
  - A soluble fraction, bound to macromolecules, which seems to stay in the tissue for a long period of time
  - An insoluble not well characterized fraction which remains in the tissue for a long period of time

- The total Gd tissue conc is too low to generate a visible MR signal (< 20 µmol Gd/L), if it is only the intact GBCA - > Gd species with high relaxivity must be present

- LA-ICP-MS demonstrated widespread presence of Gd in brain, e.g. in the granular layer and (unpublished results) other parts of the cortex of cerebellum. These areas are not visible in MRI - > These Gd species do not have an influence on T1 - > different Gd species
Gd Deposits in the Rat Brain: Laser Ablation coupled with ICP-MS of the Brain 52 weeks p.i.

- **Omniscan®**
- **Magnevist®**
- **MultiHance®**
- **Saline**
- **Gadovist®**
- **Prohance®**
- **Dotarem®**

Gd deposits in the deep cerebellar nuclei only for linear but not macrocyclic GBCAs
Brain Tissue Fractionation and Separation

Total Gd concentration in brain was very low (~0.0005% id), but higher for linear than for macrocyclic GBCAs.

No prominent difference between ionic and non-ionic linear GBCAs.

Wash out between days 3 and 24 p.i was more pronounced for macrocyclic GBCAs (-62 to -72%) than for linear GBCAs (-23 to -47%).

Differences between brain sections were minimal, with a trend for lower concentrations in pons.
Brain Tissue Fractionation and Separation

- Faster elimination of gadobenate due to additional hepatobiliary excretion of ~50% in rats, which accounts for only 3% to 5% in humans.
- About 33-60% (3 d p.i.) and 63-83% (24 d p.i.) of the Gd from linear agents was found in insoluble components.
- Basically no Gd was found in the insoluble fraction after injection of macrocyclic GBCAs (comparable to control study with spiked tissue).
- The Gd conc. of soluble components on days 3 & 24 p.i. and their elimination until day 24 p.i was similar for linear & macrocyclic agents.
- High combined recovery of Gd from both fractions (87 ± 12%), similar to the control study (94-96%).