Indirect Speciation via Kinetics

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Conflicts of Interest

Scientific Advisory Boards of Bracco Diagnostics Inc, Archeus LLC, Empirion LLC, and Enlyton LLC, and have advisory roles at Reveal LLC and Molecular Theranostics LLC. I am a Co-investigator on a grant from Geurbet. I invented the marketed GBCA, gadoteridol.

Indirect Speciation via kinetics

- Mechanism elucidation includes:
  - Identity initial state(s) (e.g. GBCA)
  - Identity of end states (e.g. $GdPO_4$, Gd-TF, etc.)
  - Identity of intermediate states (existing temporarily), (e.g. $[GBCA)_3CO_3]$, GBCA-HSA, Gd_{aq})
- *Ultimate understanding and control of reactions requires knowing How* (by what steps) species A produces subsequent species = the "mechanism"
- A limited number of reaction mechanisms (associative, dissociative, etc) exist.
- Kinetics studies can be crucial to determine mechanism. By identifying the "order" and "rate" of each step, intermediates are identified that can control the overall rate.

Hypothetical Example:

$Gd(DTPA) + Cu \leftrightarrow Gd(DTPA\text{-}Cu)$	rate = k[Cu]; Keq[Cu] = bound/free
$Gd(DTPA\text{-}Cu) + PO_4 \rightarrow Cu(DTPA) + Gd(PO_4)_s$	rate = k'$[PO_4]$
$2Gd(PO_4)_s$ + proteinX(soluble) $\rightarrow Gd_2$-protein(soluble)	rate determining step
Gd_2-protein + Cell $\rightarrow Gd_2$-protein(intracellular)	fast (t1/2 = 1h vs 24h for GBCA 1 vs 2?
Gd_2-protein $\rightarrow$ Serum and excretion	k(tissue 1) > (tissue 2)

Radiotracers are strongest as mechanism tools

- Some early biodistribution comparing ^{153}Gd-GBCA used kinetics of biodistribution pattern to suggest dechelation differences of GBCA. (Tweedle)
- Kasokat compared biodistribution in rat tissues with time for Gd(^{14}C-DTPA) and ^{153}Gd(DTPA), finding [^{14}C] > than [^{153}Gd] in tissues, proving that dechelation must have occurred. (Kasokat et al)
- ICP & ^{153}Gd radiotracers both quantitative, but ICP cannot separate by origin:
  ^{153}Gd and/or ^{14}C can be detected *independently of ^{12}C and ^{15X}Gd to study species independently* (e.g. biodistribution of ^{153}Gd + Gd(DTPA) in mice. Wedeking)
- <u>Requires "True Tracer validation" (Kumar, et al)</u>
  [radiotracer] is always very low and must be validated chemically as a genuine representative of the unlabeled bulk being investigated (a true tracer) .
  $[^{153}GdX]_{initial}$ and $[GdX]_{initial}$ must be independently known
- Less sensitive than ICP, but less labor intensive if regulatory system is in place
  - Regional or national NIH facility(ies)?

Knowledge Gap Issues

- Training in kinetics: not very common, especially in biology; mathematics describing complex systems is complex, but analysis of simple systems' kinetics provides less knowledge than for complex ones, so potential is high when the kinetics is complicated, and biological systems are always complex.

- Long lived ^{153}Gd and ^{14}C are not accurately determined simultaneously in common radioactivity counters. Experiments would be more accurate and more efficiently executed with paired data.

- Relatively few facilities with trained personnel and regulatory apparatus in place.