Developing $^{134}$Ce-Labeled Radiopharmaceuticals as Imaging Surrogates for $^{225}$Ac Compounds

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DOE IP Virtual Seminar Series – Ce-134 10.16.23
Radiosynthesis and QC

Labeling of macropa- and DOTA-containing compounds
Radiosynthesis and QC

Labeling of macro- and DOTA-containing compounds
Labeling of macropla and DOTA with Ce-134

- We compared the labeling of RPS-088, a small molecule containing macropla, and RPS-063, a small molecule containing DOTA with Ce-134 (provided in 0.1 M HCl)
- The mass of precursor was 0.5 nmol, and total reaction volume was 55 µL. The reaction was sampled from 5 min to 60 min

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Buffer</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPS-088</td>
<td>0.1 M Tris, pH 8.5</td>
<td>25 °C</td>
</tr>
<tr>
<td>RPS-088</td>
<td>0.1 M NaOAc, pH 5</td>
<td>25 °C</td>
</tr>
<tr>
<td>RPS-063</td>
<td>0.1 M Tris, pH 8.5</td>
<td>95 °C</td>
</tr>
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</tbody>
</table>

Macropa complexes > 90% $[^{134}\text{Ce}]\text{Ce}^{3+}$ within 5 min at 25 °C, while DOTA complexes > 90% within 5 min at 95 °C
QC of the radiolabeled compounds

- RCP was evaluated by radio-iTLC using two mobile phases: 50 mM citrate, pH 5 and 1 M NH₄OAc (pH 5.5):DMF = 1:1
- TLC plates were scanned 60 min after development
- In 50 mM citrate, [¹³⁴Ce]Ce-RPS-088 remains at the application point and unbound Ce-134 migrates with the solvent front
QC of the radiolabeled compounds

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- In 50 mM citrate, [¹³⁴Ce]Ce-RPS-088 remains at the application point and unbound Ce-134 migrates with the solvent front
- In 1 M NH₄OAc:DMF = 1:1, unbound Ce-134 remains at the application point and [¹³⁴Ce]Ce-RPS-088 migrates with the solvent point
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- TLC plates were scanned 60 min after development
- In 50 mM citrate, $^{134}\text{Ce}]\text{Ce-RPS-088}$ remains at the application point and unbound Ce-134 migrates with the solvent front
- In 1 M NH₄OAc:DMF = 1:1, unbound Ce-134 remains at the application point and $^{134}\text{Ce}]\text{Ce-RPS-088}$ migrates with the solvent point
- The same results are obtained when $^{225}\text{Ac}]\text{Ac-RPS-088}$ is run in the mobile phases and evaluated 2 h after plate development
QC of the radiolabeled compounds

- RCP determination by HPLC is also possible through fraction collection.
- Fractions are spotted on a grid and imaged by phosphorimager 1 h after collection.
- For small molecules, this allows radiochemical identity to be determined simultaneously.

The sizeable peak at the injection front disappears upon gamma counting, indicating that it is due to La-134.
In vitro experience

Stability studies and challenge experiments
Serum stability in human and mouse

- $[^{134}\text{Ce}]\text{Ce-RPS-088}$ was incubated at 37 °C in 100 µL human or mouse serum
- Aliquots were assayed pre- and post-precipitation of proteins up to 168 h post injection
- Stability assessed by radio-iTLC using 50 mM citrate, pH 5 mobile phase

The $^{134}\text{Ce}$-macropa complex is approximately 80% stable in human and murine serum after 72 h and approximately 25% stable after 168 h
Serum stability in human and mouse

- $[^{134}\text{Ce}]{\text{Ce}}$-$\text{RPS}$-$063$ was incubated at 37 °C in 100 µL human or mouse serum
- Aliquots were assayed pre- and post-precipitation of proteins up to 72 h post injection
- Stability assessed by radio-iTLC using 50 mM citrate, pH 5 mobile phase

The $^{134}\text{Ce}$-DOTA complex is approximately 95% stable in human and murine serum after 72 h. This complex is more stable than the $^{134}\text{Ce}$-macropa complex.
Serum stability in human and mouse – comparison to Ac-225

- $^{225}$Ac-RPS-063 and $^{225}$Ac-RPS-088 were incubated at 37 °C in 100 µL human or mouse serum
- Aliquots were assayed pre- and post-precipitation of proteins up to 72 h post injection
- Stability assessed by radio-iTLC using 50 mM citrate, pH 5 mobile phase

As expected, the $^{225}$Ac-macropa complex is significantly more stable than the $^{225}$Ac-DOTA complex. This is the inverse of the $^{134}$Ce complex stabilities
DTPA challenge experiment

- $^{225}$Ac-Ac-RPS-063 and $^{225}$Ac-Ac-RPS-088 were incubated at 37 °C with 0, 1, 100, and 1000 molar equivalents of DTPA
- Aliquots were assayed pre- and post-precipitation of proteins up to 168 h post injection
- Stability assessed by radio-iTLC using 50 mM citrate, pH 5 mobile phase

The $^{134}$Ce-macropa complex is stable up to 100x DTPA while the $^{134}$Ce-DOTA complex is stable at all concentrations of DTPA
DTPA challenge experiment – comparison to Ac-225

- $^{225}\text{Ac}\text{[Ac-RPS-063 and }^{225}\text{Ac-RPS-088}$ were incubated at 37 °C with 0, 1, 100, and 1000 molar equivalents of DTPA.
- Aliquots were assayed pre- and post-precipitation of proteins up to 168 h post injection.
- Stability assessed by radio-iTLC using 50 mM citrate, pH 5 mobile phase.

As expected, the $^{225}\text{Ac}$-macropa complex is significantly more stable than the $^{225}\text{Ac}$-DOTA complex. This is the inverse of the $^{134}\text{Ce}$ complex stabilities.
In vivo experience

Experiments in an LNCaP tumor model
microPET/CT imaging

• Mice were administered 4.5 MBq (120 µCi) of \(^{134}\text{Ce}\)Ce-RPS-088 (a macropa-containing PSMA ligand) and imaged at 2, 24, 48, and 72 h post injection
• Scan acquisition time was 30 min
• The mice were imaged with a 1% injected dose standard to enable image-based quantification of uptake
• Biodistribution was as expected, with the exception of the elevated liver uptake
• We suspect some in vivo decomplexation
Biodistribution: Intersubject comparison with $^{225}$Ac-labeled compound

- Mice were administered RPS-088 labeled with either Ce-134 (3.5 MBq) or Ac-225 (50 kBq)
- The mice were sacrificed at 24 h post injection
- Tissues were counted 24 h after collection and uptake quantified by comparison to a 1% injected dose standard

A key difference is evident in the kidneys. Our working hypothesis is that the decomplexed Ce-134/La-134 clears via the hepatobiliary pathway faster than 24 h and is not captured by this time point.
Biodistribution: Intrasubject comparison with $^{225}\text{Ac}$-labeled compound

- 2 Mice were co-administered $[^{225}\text{Ac}]\text{Ac-RPS-088}$ (50 kBq) and $[^{134}\text{Ce}]\text{Ce-RPS-088}$ (1.5 MBq)
- The mice were sacrificed at 24 h post injection
- Tissues were counted 24 h after collection and again at 61 h, 85 h, 160 h, and 328 h after collection

As expected, the quantification of total tissue uptake is consistent at all time points
Biodistribution: Intrasubject comparison with $^{225}$Ac-labeled compound

- We used a graphical method to estimate the contribution of each compound to the total tissue activity
- Standards ranging in activity from 0-100% Ce-134 were serially measured to provide data for curve fitting
- The y-intercepts of the curves give the activity of Ce-134 at t=0 (time of sacrifice)

The fit for Ce-134 is good, with a maximum error of 5.5% at higher percentages of Ce-134
Biodistribution: Intrasubject comparison with \(^{225}\)Ac-labeled compound

- We estimated the tissue activity due to \([^{134}\text{Ce}]\text{Ce-RPS-088}\) using the graphical method.
- Contribution of \([^{225}\text{Ac}]\text{Ac-RPS-088}\) was the difference between total uptake and uptake due to \([^{134}\text{Ce}]\text{Ce-RPS-088}\).
- Slightly higher background of \([^{225}\text{Ac}]\text{Ac-RPS-088}\), likely because of the 3-5.5% error in Ce-134 quantification.

The tissue distribution is consistent between animals and with the results obtained in separate mice.
Biodistribution: Intrasubject comparison with $^{225}$Ac-labeled compound

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- Contribution of $[^{225}\text{Ac}]$Ac-RPS-088 was the difference between total uptake and uptake due to $[^{134}\text{Ce}]$Ce-RPS-088.
- Slightly higher background of $[^{225}\text{Ac}]$Ac-RPS-088, likely because of the 3-5.5% error in Ce-134 quantification.

The tissue distribution is consistent between animals and with the results obtained in separate mice.
• Both macropa and DOTA rapidly complex $[^{134}\text{Ce}]\text{Ce}^{3+}$ at 95 °C

• The $^{134}\text{Ce}$-DOTA complex is stable in serum and to DTPA challenge. $^{134}\text{Ce}$-macropa decomplexes under the same conditions. This is the inverse complex stability of Ac-225

• Administration of $[^{134}\text{Ce}]\text{Ce-RPS-088}$ to mice results in high quality microPET/CT images. Small accumulation of signal in liver hints at instability of the complex in vivo

• In vivo decomplexation of $^{134}\text{Ce}$-macropa may lead to different doses absorbed by excretory organs in comparison to $^{225}\text{Ac}$-macropa compounds

• It will likely be possible to disentangle the contributions of Ce-134 and Ac-225 when co-administered to the same animal. This may enable improved dosimetry comparisons
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Thanks to Karen Sikes and team for providing the Ce-134!