Radiochemistry for a beta tomorrow: The application of radiochemistry toward molecular imaging and therapy of cancer

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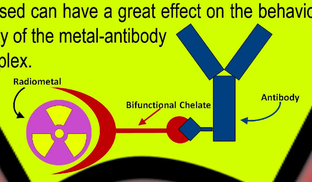
Introduction

Radiochemistry has numerous applications in nuclear medicine, primarily in helping to develop radiopharmaceuticals. The incorporation of a radioactive isotope within a known drug or as part of a new complex can be used to image or treat a disease. The difficulty lies in delivering the radiation to the desired site in the body.

Antibodies are nature's targeting vectors and the ability to use antibodies as the targeting agents for radiotracers is of great importance for both imaging and therapy. Antibodies are extremely specific targeting moieties. Various diseases, specifically different types of cancers, are known to overexpress different antigens compared to normal, healthy tissue.

In order to use antibodies as the targeting mechanism for a radiometal, one has to utilize a bifunctional chelator which can both bind the metal and conjugate an antibody. Designing an appropriate ligand for each specific radiometal is very important and the ligand used can have a great effect on the behavior and stability of the metal-antibody complex.

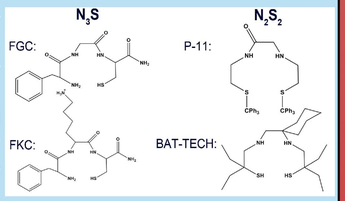


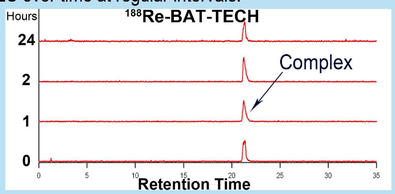
Rhenium-188

^{188}Re can be conveniently obtained from a ^{188}W/^{188}Re generator in high specific activity. It has a β_{max} energy of 2.12 MeV which is ideal for killing tumors, and its half-life of 16.9 hours is optimal to match the biological residence time of peptides and fast circulating IgM antibodies.[1] Since ^{188}Re also has a 15% gamma emission at 155 keV, it can be easily tracked in the body using SPECT imaging. The first step toward making use of ^{188}Re is to develop a bifunctional chelate that can be used to attach ^{188}Re to a targeting vector such as an antibody. The next step is to evaluate the stability of ^{188}Re chelates. Our overall objective is to develop bifunctional chelates for conjugation of ^{188}Re to 6D2, an IgM, and other antibodies.

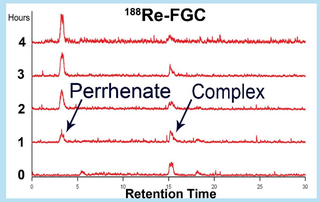
In Phase 1 clinical trials where ^{188}Re is introduced into 6D2 by direct labeling, ^{188}Re-6D2 has shown no adverse effects and in fact, target tumors were observed to stabilize or decrease in size in nearly all patients.[8]

Two families of ligands are being investigated called N_2S_2 and N_3S, where N and S represent the number of nitrogen and sulfur bonds to the central Re. While the N_3S ligands have been found to be unsuitable and quickly decompose to perrhenate, the N_2S_2 ligands, currently under investigation, are showing tremendous promise. Stability is evaluated by reinjecting HPLC purified complexes that have been dried by rotovap and reconstituted in phosphate buffer at pH 7.4 back into the HPLC over time at regular intervals.





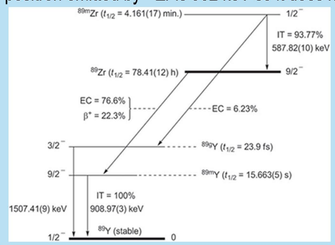
N_2S_2 ligand BAT-TECH forms a ^{188}Re complex that is still stable after 24 hours.

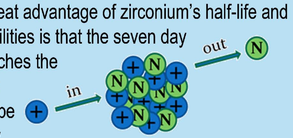


N_3S ligand FGC forms a ^{188}Re complex that is unstable after only 1 hour.

Zirconium-89

Zirconium-89 (^{89}Zr) is a radioactive isotope of with a half-life of 78.41 hours which decays to stable yttrium-89 through the emission of a positively charged beta particle (β^+) also known as a positron. The maximum energy of a positron emitted by ^{89}Zr is 902 keV so it does not travel very far in tissue before annihilating. This makes ^{89}Zr well suited for PET imaging and affords an intrinsic resolution of about 1 mm. The long half-life of ^{89}Zr allows PET images to be taken for 7 days following injection of the tracer.[4] The great advantage of zirconium's half-life and accompanying imaging capabilities is that the seven day imaging window perfectly matches the optimal circulation time of IgG antibodies. Zirconium-89 can be produced from 100% naturally abundant yttrium-89 with a (p,n) reaction in a cyclotron.

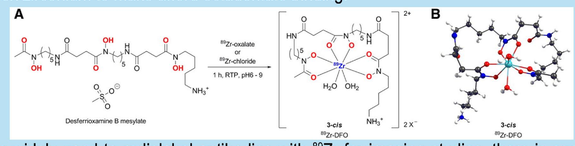




$$^{89}_{39}Y + ^{1}_{1}p^{+1} \rightarrow ^{89}_{40}Zr^{+1} + ^{1}_{0}n$$

Current Zirconium Chelation

Currently, the standard for zirconium chelation is desferrioxamine (DFO) which has three hydroxamate groups that bind the metal in a hexadentate configuration. Density functional theory (DFT) calculations suggest that two water molecules also complex zirconium to make afford octadentate binding.[5]

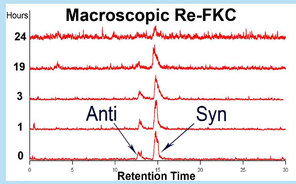


While DFO is widely used to radiolabel antibodies with ^{89}Zr for imaging studies, there is some evidence of ^{89}Zr being released from the complex in vivo. Since free zirconium is a bone seeker, this release is seen as bone uptake in mice.[6] Our pursuit of novel ligands is motivated by the desire to minimize in vivo release and accumulation of radioactive material in bone.

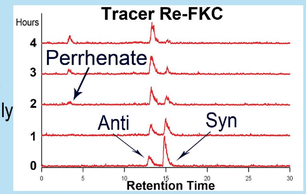
Tracer vs. Macroscopic Levels

The stability of the ^{188}Re N_3S complexes was evaluated both at the pure tracer level and when a macroscopic amount (1.34 µmol) of cold rhenium is added to the ^{188}Re reaction. The Re-FKC complex is known to have syn and anti diastereomers which can be observed by HPLC. Later measurements have significantly less signal due to decay according to the half-life of ^{188}Re of 16.9 hours.

After HPLC purification and reconstitution in pH 7.4 phosphate buffer, the macroscopic Re-FKC complex remains stable past 24 hours.



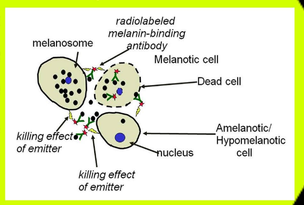
Under the same conditions, the tracer Re-FKC complex begins to decompose to perrhenate after only 2 hours. This reveals the FKC ligand to be unsuitable.

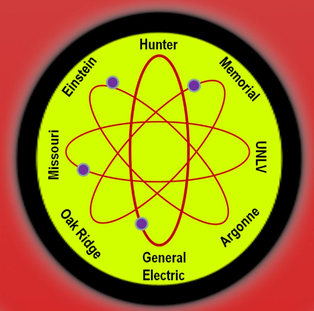


The fact that the behavior seems to be very different at the macroscopic versus tracer levels is highly significant for the evaluation and future of these ligands. It is an issue that should be considered when developing all such radiometal chelators.

Radioimmunotherapy

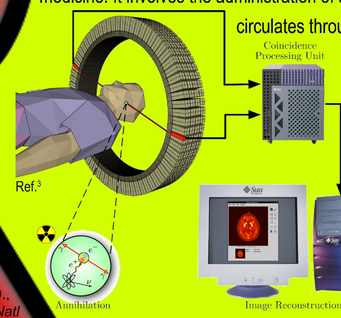
Radioimmunotherapy is the use of radiolabeled antibodies to target cancer sites and kill tumors with high energy radiation. This provides localized dose to tumors while minimizing exposure to the rest of the healthy tissue. A fast circualting IgM antibody, 6D2, targets melanin that leaks from lysed melanoma cells, and, if paired with an appropriate radioactive payload, could prove to be a very effective treatment for melanoma.





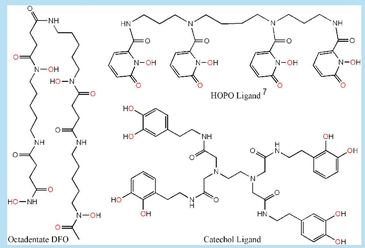
Positron Emission Tomography

PET is a non-invasive, functional imaging technique in nuclear medicine. It involves the administration of a radionuclide tracer which circulates throughout the body and accumulates in targeted areas. The selected radionuclide emits a positron and when this positron collides with an electron, they annihilate and release two simultaneous 511 keV gamma rays in opposite directions. These gamma rays are detected by rings of scintillation detectors and this data is extrapolated back to produce a 3-D image of the distribution of the drug in the body.



Novel Zirconium Chelators

In order to design a ligand for zirconium it is important to recognize that zirconium is oxophillic and requires a hard donor ligand as well as prefers octadentate complexation. With this in mind I have chosen to focus on three different functionalities as binding groups: hydroxamates, hydroxypyridinones (HOPO), and catechols. Attaching these groups to a range of linear and branched backbones will allow for the creation of a pool of ligands that can each be tested for its affinity, selectivity, and stability for binding zirconium.



Above are the three initial ligands under investigation. Synthesis has proven to be quite complex and taken longer than expected. A major concern is the need for extremely pure product in order to conduct work at the tracer level. Initial studies will be done with the base ligands and then, if they prove worthwhile, additional synthesis will be done to bifunctionalize them for further study and actual use.

Funding

NSF IGERT 0965983
NIH NCI-SC1 CA138177

Acknowledgements

Diane Abou
Kate Dadachova
Malleswari Ponnala
Shashikanfh Ponnala

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