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Evolution of Radiopharmaceuticals for Diagnosis and Therapy

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Abstract Diagnostic radiopharmaceuticals should reflect in vivo biochemistry. Therapeutic radiopharmaceuticals should kill tumor cells while sparing healthy cells. As the understanding of a biological process increases, synthetic chemists must be ever poised to improve the design of new radiotracers to reflect the in vivo scenario in an accurate and precise fashion. However, it is somewhat ironic that, due to genetic variability, there is an inverse relationship between the specificity of the radiotracer and the number of individuals who will eventually be helped by these discoveries. Regardless of the beauty of the science that underpins radiopharmaceutical development, the availability of financial resources will direct the overall progress in the field. J. Cell. Biochem. Suppl. 39: 60–64, 2002. © 2002 Wiley-Liss, Inc.

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Picture a senior synthetic organic graduate student, searching the library fruitlessly for journals where a potential employer has published papers. The time is 1981, long before the internet has made such a search a *fait accompli* with a click of a mouse. No journals were found at this library, located at a major state institution with no medical or veterinary school at the time. The advertised position, a postdoctoral fellowship, was a chance to work in Baltimore, at Johns Hopkins, in directed organic synthesis for diagnostic imaging in a field called Nuclear Medicine. It seemed like a fun thing to do. One is only young once!

It became very clear, upon learning about the field, that diagnostic imaging was undergoing a metamorphosis. Opioid receptors had just been identified a few years earlier. New knowledge was expanding the boundaries of Neuroscience. Radiotracers containing Carbon-11 (C-11), Iodine-123 (I-123), or Technetium-99m (Tc-99m) were being pursued simultaneously for these neuroreceptor studies. It was a time of great promise, great excitement in the field. Most importantly, it was a great time to be a chemist.

The radiotracer principle, for which George de Hevesy was awarded the Nobel Prize in 1943, existed as the overarching guideline for any development of new in vivo targets for external imaging. In a very short time at Hopkins, I had learned the history of technetium and firstly, the fact that technetium, being an artificially produced radionuclide, in theory, violated this basic principle of de Hevesy. Much more stringent validation studies would be required to assure that the images achieved from the signal were biochemically relevant.

Pertechnetate anion, eluted from the molybdenum-technetium generator, was used in its own right as an iodide equivalent in thyroid studies or studies of the disruption of the blood brain barrier [Richards et al., 1982]. The coordination of technetium with commercially available N,O chelating agents resulted in small, charged complexes. These complexes had been shown to be quite useful in renal studies. Adventitious binding of technetium to protein residues yielded tagged materials for permeability studies. Clinical approval of these technetium-99m agents was achieved, and they enjoyed widespread use throughout the world.

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The seemingly last frontier was obtaining a positive image of the brain. The intact blood brain barrier had long served as the impenetrable boundary that had successfully excluded technetium complexes in the chemical forms synthesized to date; i.e., either as charged or large molecules.

The goal at the time, for technetium chemists interested in the brain, was the synthesis of small, neutrally charged and lipophilic complexes. It was anticipated that these complexes, if they fulfilled these criteria, would passively diffuse into the brain [Oldendorf, 1978]. My initial project, to synthesize precursors to the fentanyl class of opioid receptors for C-11 studies, was put on the back burner to work on tetradentate chelating ligands containing nitrogen and sulfur coordinating atoms [Burns et al., 1978]. The chemical challenges of stabilizing a metal that can exist in multiple oxidation states became quite clear.

Groups attempting to reach this goal at this time focused on one of two major ligand systems: the N_2S_2 diaminedithiol tetradentate ligands (DADT, BAT) and the propyleneamineoximes (PnAOs). We were focused on the N_2S_2 ligands as was the Kung group [Kung et al., 1983], while Troutner and Volkert were focused on the PnAOs [Troutner et al., 1984]. As anticipated, each parent ligand structure formed a neutral complex with technetium. Each technetium complex was shown in animals to cross the blood brain barrier. However, after the passive diffusion of the complex across the blood brain barrier, it was found that each complex then diffused easily from the brain—there was no retention mechanism built into either complex. Because the clinical hope of these radiotracers was quite high, industrial collaborations were in force during this time. Structural modifications on the ligand systems took different approaches. With the N_2S_2 ligands, chemical derivatization to include amine groups proved to help retain the technetium complex for a short period of time [Lever et al., 1985]. In contrast, the PnAO ligand was modified with the addition of methyl groups to destabilize the complex. In vivo, it was found that the portion of the lipophilic complex that entered the brain decomposed and the radioactivity was trapped inside. The imaging instrumentation at the time could record the location of the radioactivity, and there was little change in the distribution of the tracer activity for sufficient time to permit successful imaging. The product of these studies was approved for use in humans and marketed as Ceretec®. Detailed structure-activity studies, previously relegated to purely organic molecules, were performed on each class of complexes. Optimum features of the complexes were found to include the p*K*a of the amine-derivatized side chain N_2S_2 ligands and the overall lipophilicity of the complex [Hui et al., 1991].

The chemical stability of the N_2S_2 ligands was combined with enzymatic hydrolysis of a side chain by chemists at NEN-DuPont to form a complex that achieved the same "photograph" quality [Walovitch et al., 1989]. This compound has been marketed as Neurolite®. The clinical utility of brain perfusions agents such as Ceretec and Neurolite are now well established. However, one novel application, where Ceretec is utilized to observe brain activity in Tibetan Buddist meditators, is clearly unique [Newberg et al., 2001].

While the goal of perfusion imaging was being pursued in the area of technetium chemistry, neuroreceptor imaging with carbon-11 and iodine-123 was making great strides delving into the myriad mysteries of the brain. Whereas *N*-methylspiperone is an achiral molecule [Wong et al., 1984] and diprenorphine [Lever et al., 1987] is a semi-synthetic opioid ligand derived from a chirally pure natural product, molecules like iodo-quinuclidinylbenzilate (I-QNB) [Rzeszotarski et al., 1984] required enantiomerically pure substrates to be most effective.

In the medical arena, however, many approved medications are supplied as their racemic mixtures. Methylphenidate, prescribed for the treatment of attention deficit disorder is one of these medications. Labeling the more active enantiomer of methylphenidate with carbon-11 ([C-11]-D-threo-methylphenidate) was accomplished at Brookhaven National Lab [Ding et al., 1995]. Results indicated that non-invasive imaging in baboon brain could discern the differences in distribution of this more active enantiomer. These results support the ever-evolving concept that imaging can be useful in drug development.

Naturally, compounds analogous to carbon-11 and iodine-123 tracers were desired for technetium-99m. Extension of the synthetic organic chemistry for these new goals, to provide highly functionalized suitable ligands

for technetium complexation, was in its infancy. Clearly, these goals qualified for the "high risk, high reward" category. In addition, to the basic chemical challenges of synthesis, many pharmacological requirements placed constraints on the targets to be synthesized. The chemical properties of overall molecular weight, lipophilicity, and ionization profile [Hansch et al., 1987] were factors that constrained the synthetic approaches. In the area of N_2S_2 ligands, synthetic approaches had to address the incorporation of multiple functional groups into the molecule, as well as retain the basic affinity for the receptor. Staging the synthetic steps to be compatible with the oxidation/reduction status of sulfur, nitrogen and oxygen containing moieties proved to be quite challenging.

Progress in this field has been made for the dopamine transporter. Kung et al. reported on TRODAT, an N_2S_2 derivatized tropane molecule [Kung et al., 1996], while Madras et al., reported on the utilization of Technepine [Madras et al., 1996]. The most interesting fact about each of these molecules to this chemist is that the attachment of the chelate onto the tropane skeleton is at different positions, but both of the resulting complexes maintain high affinity. In addition, the tropane fragment itself is quite small relative to the Tc-complex; however, the overall complex in each case maintains affinity and in vivo activity. In a recent article [Johannsen and Pietzsch, 2002], the advances in technetium-based complexes for CNS applications have been reviewed. In their assessment, it is interesting to note that the most critical feature for success in vivo remains the effective transfer of the complex across the blood–brain barrier.

Concurrent with achieving the preparation of Tc-based compounds for the central nervous system (CNS) applications, the technetium field was also immersed in the goal of labeling proteins and antibodies. Most metals, incapable of forming strong bonds directly with residues found in proteins prompted the development of the bifunctional chelate approach [Sundberg et al., 1974]. The large-scale production of antibodies [Kohler and Milstein, 1975] provided a ready source to biological targets that would provide new found specificity for in vivo situations.

At the same time, many scientists in the field had been investigating the use of radiopharmaceuticals not only as diagnostic imaging agents, but also as radiotherapeutic agents. For those involved in technetium chemistry, rhenium-186 and rhenium-188 (Re-186/Re-188) were being promoted as therapeutic analogs to technetium-99m [Deutsch et al., 1986]. One critical impediment lay in the path of potential success of radiometal labeled antibodies: chemical conditions for incorporation of the chelate or the metal usually caused loss of immunoreactivity in the antibody. New chemical methods were needed. For the incorporation of technetium and rhenium, the preparation of a "ready-made" bifunctional chelating agent proved quite useful [Baidoo and Lever, 1990]. Coupling of the reactive thiolactone moiety to the ε-amino group of a lysine residue could be accomplished quickly at room temperature and at a pH compatible with the antibody. Covalent coupling also resulted in the release of the arm to complete the DADT tetradentate chelate. Subsequent incorporation of the radiometal was accomplished in a facile manner.

Besides the Tc/Re diagnostic/therapeutic pair, indium-111 and yttrium-90 (In-111/Y-90) also existed as a matched diagnostic/therapeutic pair. Chosen chelates for these metals were primarily the polyaminocarboxylates; e.g., diethylenetriaminepentaacetic acid (DTPA) derivatives. The open-chained ligands provided quick labeling kinetics with radiometals; however, the ligands did not provide optimum in vivo stability. Synthetic chemistry revolved around constraining the open-chained ligand, which improved the in vivo stability of the radiometallated complex [Brechbiel and Gansow, 1991]. Zevalin®, an antibody coupled to a bifunctional chelating agent and radiolabelled with Y-90 was a result stemming from this research, and was approved as the first radioimmunotherapeutic agent [Wiseman et al., 2002]. Radiotherapy became very important for many groups. In addition to constrained open-chain ligands, macrocyclic polyaminocarboxylates, most notably 1,4,7,10-tetraazacyclododecane *N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) was identified for the stable complexation of radiometals [Moi et al., 1998].

In addition to rhenium and yttrium, other radiometals for therapy emerged. Of these, samarium-153 (Sm-153) became the active ingredient in Quadramet®, a product approved for the pain palliation of bone metastases [Goeckeler et al., 1987]. Sm-153, produced at

the University of Missouri Research Reactor (MURR) has been joined by the radiolanthanides of holmium-166 (Ho-166), lutetium-177 (Lu-177), and promethium-149 (Pm-149) as useful radiotherapeutic radionuclides. The potential that exists for these radionuclides in cancer therapy prompted a move from Johns Hopkins to the University of Missouri at Columbia. MURR, the home of the largest academic research reactor, has had a rich history in the safe, reliable production of radionuclides for biomedical research.

The method of attaching a chelate to a biological target for incorporation of the radioactive metal is quite commonplace at this time. Drawbacks in the in vivo localization of antibodies spurred interest in techniques that would permit pre-concentration of the antibody at the target site, followed by small radiolabeled complexes that would accumulate at the binding location of the antibody. This technique, termed pretargeting, is an elegant approach to a difficult delivery issue [Goodwin and Meares, 1997]. Another method to obviate the need for the use of radiolabeled antibodies was the radiolabeling of high affinity peptides. Octreotide, a fragment of somatostatin, has been modified for incorporation of metals for diagnostic and therapeutic applications [Bakker et al., 1991] Building upon Bruce Merrifield's accomplishments, for which he was awarded the Nobel prize in 1984, solid phase peptide synthesis has since been automated and can provide ready access to octreotide derivatives as well as new substrates. Sufficient in vivo stability of newly identified endogenous peptides is usually lacking for extended residence time required for successful radiotherapy. Here, too, synthetic input is still required to stabilize these peptides, incorporate the chelating group, and radiolabel with the chosen metal.

Identification of the appropriate biological targets is important, because markers that act as "surrogates" under study for in vivo biochemistry are losing favor. Phage display, as a means to identify biologically relevant entities, holds much promise [Smith and Petrenko, 1997]. The preparation of new radiopharmaceuticals for diagnosis or therapy will evolve only if close interactions between biologists, chemists, and physicians continue. As new biological targets are identified, the synthetic challenges presented to the radiopharmaceutical chemists will be faced and mastered.

One complicated issue that will impact the evolution of new radiopharmaceuticals for diagnostic imaging and radiotherapy is that the financial costs to validate a radiotracer through Phase III clinical trials continue to rise dramatically. Regardless of the beauty of the science involved in the development of the radiotracer, the ultimate goal is not the science, but the ability to improve the quality of life. Therefore, the current challenge for those individuals interested in this field is to achieve a balance between the specificity of a radiotracer, the required validation of the radiotracer, and the number of patients the radiotracer can help. A full understanding of the potential clinical applicability should be a mandatory requirement of any basic science proposal. Only in this way will a cohesive program for the future development of new radiopharmaceuticals be possible.

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