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HISTORICAL PERSPECTIVE

Unparalleled Contribution of Technetium-99m to Medicine Over 5 Decades

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Since the first tracer study by George C. de Hevesy in 1923, in which he used the tracer principle to study the absorption and translocation of lead nitrate with Pb-212 (1), radionuclides have been used both in vitro and in vivo to trace various biologic processes. The radioisotopes of iodine in particular have played an important role in the early development of nuclear medicine since their use in thyroid metabolism studies (I-131) and radioimmunoassay (I-125). Although I-131 did not have ideal nuclear properties for external imaging with the Anger camera, it was used extensively because of its ready availability and easy conversion to the appropriate chemical form. A long list of I-131–labeled radiotracers illustrates its importance in the postwar period (2). However, the rapid succession of discoveries of the element technetium (Tc), the molybdenum-99 (Mo-99)/Tc-99m generator (Fig. 1) and the "instant" kit (Fig. 2) concept led to unparalleled growth in nuclear medicine (3). Tc-99m radiopharmaceutical imaging is a key diagnostic in ~85% of nuclear medicine procedures. There are about 40 million procedures performed worldwide per year, which includes 20 million procedures in North America.

The early history of technetium and technetium radiopharmaceuticals is best characterized by the quote from Louis Pasteur: "In the fields of observation, chance favors only the prepared mind" (4). In the mid-1930s, the periodic table of Mendeleyev had a missing element to the right of Mo and above rhenium. During a visit to the Radiation Laboratory at the University of California, Berkeley, California, Emilio Segre obtained metal pieces of a cyclotron deflector, which was made of Mo, and on his return to Italy investigated the composition of the radioactive metal. He thought that, since the Mo was irradiated with deuterons, the missing element 43 might be present. Together with an analytical chemist, Carlo Perrier, at the University of Palermo, Palermo, Italy, he discovered the element technetium working by analogy using chemical reactions that were known for rhenium (5). Shortly, thereafter Segre and Glenn Seaborg discovered Tc-99 and Tc-99m (6).

A dramatic change for nuclear medicine occurred in the early 1950s at Brookhaven National Laboratory (BNL), Upton, New York, which brought the recently discovered element into medical practice. The laboratory was involved in the chromatographic separation of fission products and their possible applications for the peaceful use of the atom. The radioisotopes of iodine had been used in medicine for some time so they were the first focus of the laboratory research. In the process of separating the daughter radiation, I-132, from the fission product Te-132, the I-132 was found to be contaminated with Tc-99m. This observation and subsequent experiments led to the development of the Mo-99/Tc-99m generator (7). When the long-lived parent Mo-99 ($t_{1/2}$ = 66 h) was adsorbed to alumina, the short-lived daughter Tc-99m ($t_{1/2}$ = 6 h) could

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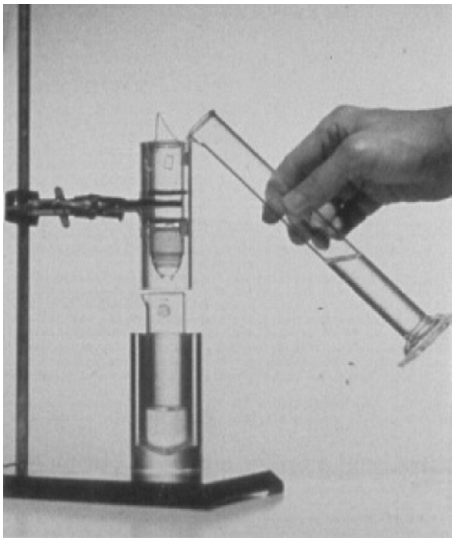

Figure 1. The Original Mo-99/Tc-99m Generator (Shown Without Lead Shielding)

The parent molybdenum-99 (Mo-99) is adsorbed to a small amount of alumina. Pertechnetate is not adsorbed to the alumina and can be eluted with a small volume of saline. This provides a ready source of the 6-h half-life technetium-99m (Tc-99m) in the clinic. Reprinted with permission from Richards (7).

be eluted with isotonic saline (8). Of the conveniently available radionuclides, including the often-used I-131, Jim Richards thought that technetium had by far the best nuclear properties for imaging with the Anger camera because the 140-keV gamma emission had satisfactory tissue penetration (50% is absorbed in 4.6 cm of tissue), yet the energy was low enough to be collimated easily. In this case, the imaging device was already in place and together they produced quality external imaging of the biodistribution in vivo.

During the mid-1960s, I was in graduate school studying the reaction of hot (energetic) silicon with organic molecules, an early example of molecular targeting that some thought was in anticipation of a silicon-based life form. After a short stay at Mallinckrodt Nuclear the successor to Wil Konneker's Nuclear Consultants, Inc., working on the challenges of low-yield generators, I left for BNL. At BNL, Jim Richards and I often discussed expanding the use of Tc-99m; it was clear that a further advance in the chemistry would be necessary before Tc-99m was used widely. The chemical form of Tc-99m eluted from the generator is pertechnetate (TcO_4^-), the most stable chemical state of technetium in aqueous solution (9). But pertechnetate could not be used directly because it does not bind to chelating agents or directly to biological molecules. Consequently, a less stable lower oxidation state of Tc-99m must be formed using reducing agents. In the reduced state, technetium binds readily to chelating agents, thereby forming such compounds as Tc-99m diethylenetriaminepentaacetic acid (DTPA), Tc-99m human serum albumin, and Tc-99m–labeled red blood cells, which turned out to be our early targets and the subject of a patent.

What reducing agent was used for the first studies? Many radiopharmaceuticals were formed using ferric chloride and ascorbic acid as the reducing agent (10). Although this produced a suitable radiopharmaceutical, the need to use special equipment, such as a pH meter, and to prepare sterile buffers prevented the routine use of Tc-99m radiopharmaceuticals in most nuclear medicine laboratories. No other "contrast media" used in radiology required this much in-house preparation, and therefore Tc-99m was destined to be an interesting but little-used radionuclide. The introduction of stannous ion as a reducing agent permitted pertechnetate to be added to a vial containing the targeting agent with no further pH adjustment or addition of other chemicals. The reaction was deceivingly simple; pertechnetate is added to a vial, reduced at pH 4 to 7, and then bound by the chelating agent (11). On a practical level, the use of stannous ion was a key development and current radiopharmaceutical

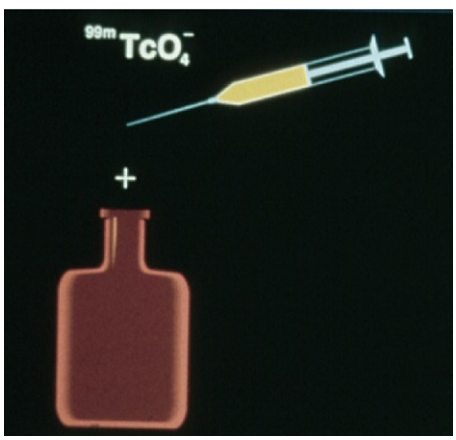

Figure 2. The "Instant" Kit Formulation

Pertechnetate, eluted from the generator, is added to a vial containing a reducing agent (stannous ion) and a targeting agent (such as diethylenetriaminepentaacetic acid) formulated such that upon the addition of pertechnetate, the Tc is reduced and bound to the diethylenetriaminepentaacetic acid in high yield. Although the procedure is deceivingly straightforward, Tc must be reduced and bound to the chelating agent with <5% radiochemical impurities such as pertechnetate and reduced colloidal Tc.

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kits employ the stannous reduction technique. With the advent of the Mo-99/Tc-99m generator in the 1960s (11) followed by the development of "instant" kits (12), the use of Tc-99m–labeled compounds expanded rapidly.

Because a stable or long-lived isotope of Tc (Tc-99) was not available until much later, the characterization of the structure and oxidation state of these early radiotracers was carried out using approaches similar to those Segre and Perrier used to first identify the element. In collaborations with Joseph Steigman and Jim Richards, we carried out experiments to determine the oxidation state and chemical form by observing the similarities with well-known Re chemistry (13).

With the availability of long-lived Tc-99 (there is no stable isotope of Tc) crystal structures were elucidated for Tc radiopharmaceuticals. The publication of the first Tc crystal structures of potential radiopharmaceuticals appeared simultaneously in 1978. In one, pertechnetate was reported to have reacted with an impurity in thioglycolic acid to produce oxotechnetium bis (thiomercaptoacetate) anion (14). In the other seminal effort in inorganic chemistry, pertechnetate was reduced by sodium borohydride in ethanol in the presence of 1,2- or 1,3-dithiols, and the products were isolated as the Tc bis structures (15).

After a stay at BNL, I moved to George Washington University (GWU), Washington, DC, to join Dick Reba, where 2 choices we made for targeted molecules illustrate the challenge with Tc-99m. We choose to radiolabel quinuclidinyl benzilate (QNB), a muscarinic antagonist with I-123 because of QNB's relatively low molecular weight. We also radiolabeled palmitic acid with Tc-99m. 4-[I-123]IQNB became the first ligand to map mAChR in a human using external imaging (16). On the other hand, Tc-99m palmitic acid did not retain its biological activity. Although satisfactory heart-to-blood ratios and expected metabolic profiles have been observed over the past 30 years for various Tc-99m fatty acids, the radiolabeled fatty acids have not been shown to follow the biochemical pathway of palmitic acid (17). This illustrates the difficulty of incorporating not only Tc-99m but its required chelating agent as well into relatively small molecules. A limited number of small molecules with a molecular weight of $<$500 Da have been radiolabeled and found to retain their biological activity, namely Tc-99m TRODAT for monitoring dopamine transporter densities (18) and a Tc-99m lisinopril analog that monitors angiotensin-converting enzyme densities (19). But it is a challenge, considering the number of successful I-123 small molecules that have retained an important aspect of the biological activity of the parent compound.

The development of Tc-99m myocardial perfusion agents has an interesting history. In the heart, ^{201}TI as the thallous cation was most often used as a potassium surrogate to measure myocardial perfusion. The original goal was to produce a Tc-99m radiotracer with a positive charge that would follow the biochemical pathway of the thallous cation. Although ^{99m}Tc cations, such as hexakis (2-methoxy-2-methylpropyl) isonitrile (sestamibi) (20) and 1,2-bis[bis(2-ethoxyethyl) phosphino] ethane (tetrofosmin) (21) were taken up in the myocardium, the mechanism of action was not related to potassium or thallium biochemistry. Yet their extraction fraction was such that they were useful radiopharmaceuticals for measuring myocardial perfusion.

After my stay at GWU and a brief foray into positron emission tomography (PET) at the National Institutes of Health, Bethesda, Maryland, I moved to the Squibb Institute for Medical Research, New Brunswick, New Jersey, as the Vice President for Diagnostics. During that time, we brought another myocardial perfusion agent to the clinic, namely Tc-99m–labeled teboroxime, a neutral compound that is taken up rapidly in the heart and released rapidly from the heart (22). We proposed teboroxime as a single photon analog of the PET radionuclide Rb-82, which has a half-life of 1.25 min. The former has a short biological half-life and the latter has a short physical half-life. At the time, myocardial perfusion studies were being carried out using Rb-82 and PET cameras that encircled the patient. Although the extraction approached 100% for teboroxime and is unequaled in that aspect among radiopharmaceuticals, the net efflux from the myocardium degrades the linearity of the relationship of uptake with flow. Just as the Anger camera was not ideal for I-131, the rotating single-photon emission computed tomographs (SPECT) of the day were not fast enough to measure perfusion with teboroxime in a matter of minutes to take full advantage of the high extraction. Some ~20 years later, SPECT is now approaching that capability. The necessary interplay between the imaging device and the radiopharmaceutical has been a constant over the years.

What is the future of Tc-99m? The strength of nuclear medicine is the tracer principle (1) and, when combined with the targeting of low-density sites using the "magic bullet" concept of Ehrlich (23), confers a unique quality to radiopharmaceuticals. Tc-99m is best as a radiolabel for peptides given that the Tc-99m chelate does not affect the biological activity if placed appropriately in the peptide and the pharmacokinetics of peptides best match the physical half-life of Tc-99m (24). The peptides studied to date are many: somatostatin, VIP/PACAP, bombesin, CCK-B/gastrin, neurotensin, alpha-MSH, neuropeptide Y, GnRH/LHRH, substance P, and opioids (25). These Tc-99m–targeted radiotracers are used for monitoring the change in target density as a function of disease progression or treatment. They also have been employed in the burgeoning field of biomarkers for pharmaceuticals (26). Certainly, the rapid growth of Tc-99m in nuclear medicine after the discovery of the element in the late 1930s is unparalleled. With the generator in the late 1950s and instant kits in the late 1960s, a medical discipline was established with the staying power to be a major diagnostic technique over the past 5 decades.

Likewise, what is the future of the generator? Do we need the generator, especially in these times of unreliable sources of Mo-99 from a limited number of aging reactors around the world and the low probability that new reactors will be built (27)? Will accelerator production of Mo-99 via photo-fission using an electron accelerator (28) or via a cyclotron-based spallation neutron source produced by proton irradiation of a Pb-Bi target (ADONIS) (29) replace reactor production of Mo-99?

Or should the direct production of Tc-99m on an accelerator using Mo targets (30,31) replace reactor production of Mo-99? Since the development of the Tc-99m generator in the 1950s, radiopharmacies have increased and shipment of the shorter half-life F-18 in final chemical form has become routine so perhaps a direct shipment of either pertechnetate or the final Tc-99m radiopharmaceutical should be the paradigm. It is unlikely that Tc-94m will supply a PET answer to the use of Tc because of the short half-life and radionuclidic impurities (32). Or as the field of targeted imaging moves to smaller molecules is the use of I-123 going to expand? These are questions that many scientific organizations are addressing in terms of capital investments, cross sections, production costs, waste disposal, and distribution requirements. Few products have avoided cannibalization in this era of discontinuity (33). The Tc-99m generator has been reinvented on several occasions to address the need for high specific activity Mo-99, for maintenance of oxidized Tc in the form of pertechnetate in the presence of generators that continue to increase in total radioactivity, and for yet smaller alumina columns and therefore less shielding. Will it continue to stand the test of time as few products have in the history of business?

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