

Optimising Short-Lived Isotopes for Quantitative Whole-body Autoradiography in Drug Development



Even the most reliable tools in the research laboratory can benefit from continued refinement. Radioisotopes have long been a dependable means for studying the absorption, distribution, metabolism, and excretion (ADME) of drug candidates in non-clinical studies. Over the years, new technologies have made better use of these radioisotopes. Quantitative whole-body autoradiography (QWBA), which has brought many improvements to the realm of tissue distribution, has itself been improved recently by the expanded use of short-lived isotopes. This expansion increases options for better imaging and more specific data regarding tissue distribution due to increased resolution of QWBA images as compared to PET or SPECT images.

Radioisotopes in Tissue Distribution

Radioisotopes, also known as radionuclides, are atoms with an unstable combination of protons and neutrons. This instability causes the atoms to give off radioactivity in the form of gamma rays or beta particles. Radioisotopes are common in daily life, finding use in smoke detectors (Americium-241), electric blanket thermostats (Promethium-147), and radio-carbon dating of dinosaur bones (Carbon-14).

Certain radioisotopes are valuable in discovery, and non-clinical and clinical drug development, as a means of tracking how therapeutic molecules interact with biological systems. They can help researchers identify distribution or efficacy issues early in a compound's development life, an issue that remains critically important as drug discovery and development becomes increasingly complex and expensive. Use of radioisotopes dates back to the 1920s, when George de Hevesy¹, a Hungarian radiochemist at the University of Freiburg (Germany), conducted studies with radionuclides to study metabolic pathways in rats. A compound is tagged

with a radioisotope, administered to an animal or human, and studied by various means as it makes its way through the body, including how the compound is distributed in tissue.

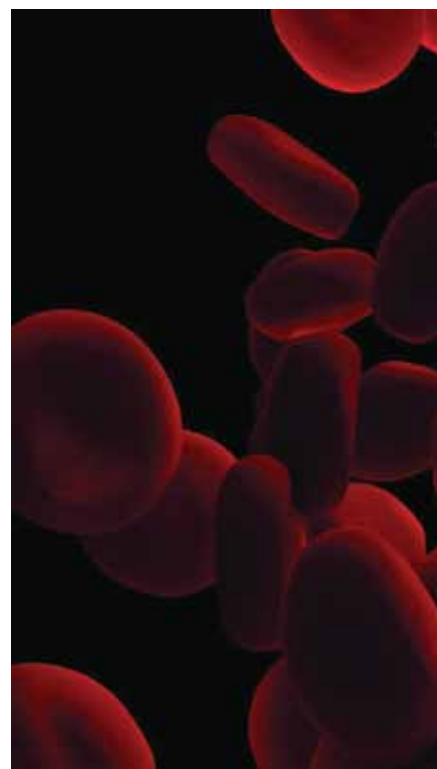
There are three common methods of determining tissue distribution of drug candidates with radioisotopes:

- **Tissue excision.**

After administering a compound to an animal model, entire tissues or organs are excised as a function of time, homogenised with water or buffer, and weighed aliquots of the homogenate are analysed. For gamma emitters, samples can be counted directly via a gamma counter. These samples are collected based on time points specified in the study protocol that are related to the pharmacokinetic profile of the therapeutic agent under study. This typically involves three animals per time point, for statistical relevance, with terminal necropsy required to excise the tissues.

- **Quantitative whole-body autoradiography.**

As the name indicates, QWBA provides a whole-body image that shows the tissue distribution of total radioactivity while distinguishing the regions of tissues or organs in which the radioactivity is found. Typically conducted with one animal per time point, animals are euthanised at specified time points and frozen in a hexane/dry ice bath. After freezing, the carcass is frozen in a block of carboxymethylcellulose (CMC) solution and cut into thin sections using a cryomacrotome. Sections that are approximately 40 μm thick are collected from the sagittal plane and captured on adhesive tape. Appropriate sections selected at various levels of interest are collected to encompass all major tissues and organs. By keeping the entire animal intact, discrete anatomic regions of tissues may be distinguished – kidney



medulla versus cortex, for example, or spleen red versus white pulp. Additional advantages include multiple data points within the same tissue or organ, lessened risk of contamination in sampling, and fewer animals needed. QWBA can be conducted in conjunction with other ADME functions, by collecting blood and excreta, for absorption and routes of excretion, or as a stand-alone procedure solely for tissue distribution.

- **Molecular imaging.**

Use of positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are more recent developments. PET and SPECT provide three-dimensional images of how radioisotopes are distributed in tissues, as well as a complete time course for ADME in the same animal. Molecular imaging provides a good first look at tissue distribution of isotopes. Both imaging techniques are widely used in the medical field to study metabolic activity.

Utility of Short-lived Isotopes

The radioactive decay of an isotope is measured in half-life, the average length of time required for a substance to lose half of its radioactivity. This rate of decay can vary widely between isotopes, from milliseconds to millions of years.

Carbon-14 (^{14}C) – that of the aforementioned dinosaur bones – has traditionally been the isotope of choice for ADME studies because it has an exceptionally long half-life, some 5,730 years, and thus presents no risk of negatively affecting a study's analysis due to loss of radioactivity through decay. Since the collection of meaningful data can be limited by a very short half-life, a longer half-life can be beneficial. However, ^{14}C does have disadvantages. Because of its relatively low energy, more of the isotope is needed in order to supply measurable activity in the tissues.

On the other hand, short-lived isotopes typically deliver a more concentrated energy signature in a shorter period of time. The shortened half-life of these isotopes greatly reduces disposal costs as they can simply be stored for 10 half-lives, allowing the radioactivity to decay to background levels which can then be disposed of in the regular trash – after the appropriate confirmatory wipe tests. Most of these isotopes are used clinically and are relatively abundant.

Of course, short-lived isotope is a relative term. (Carbon-14 is “short-lived”

compared to Iodine-129, which has a half-life of nearly 16 million years.) For the purposes of this article, “short-lived” refers to isotopes with a half-life of days or hours. The ideal is to select an isotope that can be attached to a compound effectively, radiates at an adequate energy level to accurately determine tissue distribution, and has a half-life close to the time needed to complete the study.

PET imaging can capture a single isotope type to a resolution of about 1.2 millimetres. PET is used in rodent models, with a typical dose of 250-500 microcuries (μCi) per rodent. SPECT is capable of imaging multiple isotopes to a resolution of about 0.8 millimetres, with models including rodents (typical dose up to 1.5 millicuries, or mCi) and non-human primates (up to 4 mCi).

With the benefits of using short-lived isotopes in molecular imaging understood, expanding their use to quantitative whole-body autoradiography offers great potential in streamlining and improving non-clinical ADME studies.

Optimised for QWBA

The biggest challenge to using isotopes with shorter half-lives is time constraint. If study duration is longer than the marker's half-life profile, the isotope will decay to levels of radioactivity below the limit of quantitation before the study concludes.



We began with an *in vitro* study using isotope-spiked standards to determine proper phosphor screen exposure duration for each isotope. Eight exposure time points were selected between two and 96 hours, with each isotope yielding varying results. Each isotope from each exposure was evaluated for variables such as background radiation, intensity of signal, and number of visible standards within acceptable ranges. After the evaluation, an optimal exposure duration was chosen for each isotope.

The next step was *in vivo* studies involving rodent models, using QWBA in concert with PET or SPECT. When using short-lived radioisotopes, the QWBA process differs slightly from the standard approach. In both cases, animals are dosed and euthanised at a specified endpoint. Specimens are then frozen in a hexane/dry ice bath, blocked in CMC, and sectioned using a cryomacrotome.

In the revised process, sections are collected at both $20\mu\text{m}$ and $40\mu\text{m}$, with the thinner sections freeze-dried for 24 hours (as opposed to 36-48 hours in the standard process). Depending on the half-life and energy output of the isotope, the amount of radioactivity on a particular section should be balanced with how long it takes to freeze-dry.

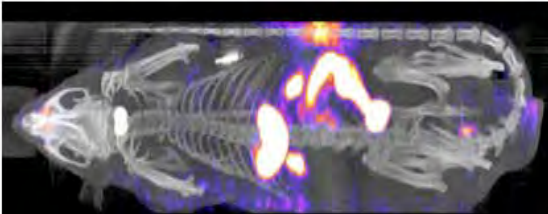
Phosphorimaging screen exposure time will vary depending on the isotope used. Care must be taken to manage co-exposure with other sections; we used lead shielding between screen cassettes in a lead box to avoid contamination. The phosphor screen is scanned and an image is produced for analysis.

Isotope	Notes	Half-life (Days)	Gamma (KeV)	Imaging Modality
Fluorine-18 (^{18}F)		0.076	511	PET
Technetium-99m ($^{99\text{m}}\text{Tc}$)		0.25	141	SPECT
Copper-64 (^{64}Cu)	Good for peptide and protein labelling (peptide is bound to diethylenetriaminepentaacetic acid (DTPA) or tetraazacyclododecane tetraacetic acid (DOTA))	0.529	511 7-8	PET
Iodine-123 (^{123}I)	Similar to Iodine-125 with a shorter half-life	0.547	159 27	SPECT
Indium-111 (^{111}In)	Good for peptide and protein labelling (peptide is bound to DTPA/DOTA)	2.8	245 171	SPECT
Thallium-201 (^{201}Tl)	Used in gated studies of cardiac muscle	3.04	71	SPECT
Iodine-124 (^{124}I)	Similar to Iodine-125 with a different energy range	4.18	511 603	PET
Iodine-125 (^{125}I)	Good for peptides, proteins, and antibody labelling	60.14	35.5	SPECT

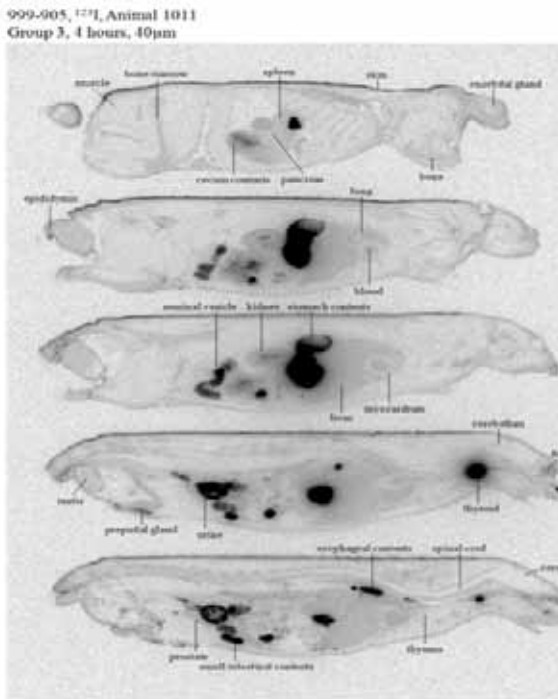
For this *in vivo* stage, ^{123}I (Figures 1a and 1b), ^{111}In (Figures 2a and 2b), and ^{64}Cu (Figures 3a and 3b) are used and each proved to have unique attributes that would prove useful in a variety of studies:

Figures 1a and 1b: Uptake of ^{123}I was found in the thyroid and stomach, with elimination via urine. Thyroid uptake was particularly large because no potassium iodide was added to the rodents' water.*

1a

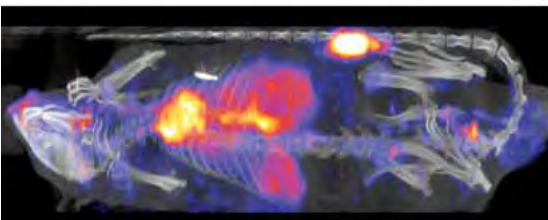


1b

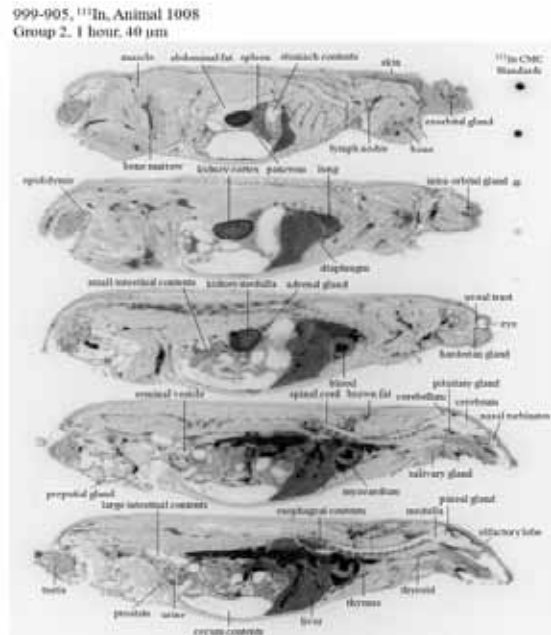


Figures 2a and 2b: Post-dose images were taken at one, four, eight, and 24 hours, yielding quality images for ^{111}In . Wide distribution was noted throughout the entire animal, with a large quantity of radioactivity found in the blood and kidney.*

2a

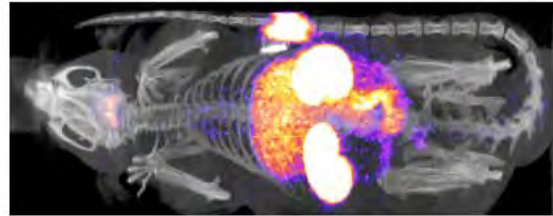


2b

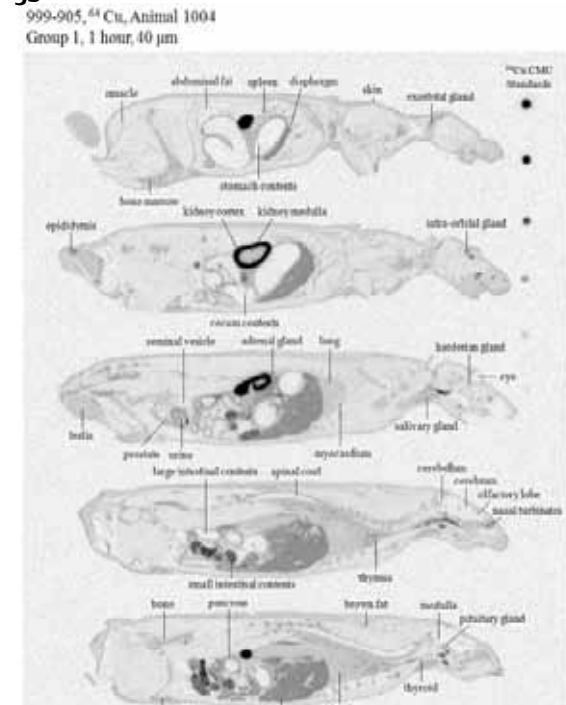


Figures 3a and 3b: Tissue distribution of ^{64}Cu mostly involved the kidneys, liver, and gastrointestinal tract, with elimination via faeces. QWBA concentration data correlated extraordinarily well with PET concentrations data.*

3a



3b



* All images produced at MPI Research, Mattawan, MI

Evolving Better Applications

Traditionally, QWBA has relied on ^{14}C , ^3H (Tritium), and ^{125}I . Each of these longer half-life isotopes has its advantages; for example, ^{125}I is frequently used in studying large molecules, and ^{14}C remains a standard among long half-life isotopes. Now, given the positive results generated through QWBA and molecular imaging using short-lived isotopes, drug developers can expand the application of these tools to maximise data generation and reduce the number of animals used.

To that end, we continue to identify and test isotopes to further equip the QWBA toolbox. To date, we have optimised ^{123}I , ^{64}Cu , ^{111}In , ^{201}Tl , $^{99\text{m}}\text{Tc}$, and ^{18}F , meaning we have a complete picture of optimal exposure times and standard curves. We are now pursuing studies with ^{124}I , Strontium-85 (^{85}Sr), and Calcium-45 (^{45}Ca), with an eye on testing other candidate isotopes as opportunities arise.

Short-lived isotopes were once considered unsuitable for QWBA. As noted, time constraints have been the biggest challenge – a challenge that can be surmounted through careful avoidance of process delays and in the selection of isotopes based on the timeline needs of a particular study. This is where close communication between the various disciplines involved in a study is paramount so that the process is clearly understood and any anomalies can be identified quickly. Balancing the decay half-life of an isotope with the pharmacokinetic profile of the compound is important as

well; a drug candidate with a longer half-life of elimination from the animal will either require an isotope with a longer half-life or a higher dose of radioactivity.

Our research has shown that, with proper adjustments in the process, use of short-lived isotopes is quite feasible in this setting. Further, pairing QWBA with another molecular imaging modality can provide a “bigger picture” into the distribution of the compound being studied. Generating more data quickly, reliably, and cost-effectively is the mantra

of modern drug development. Enhancing the tools at hand, including more effective use of imaging techniques, remains an area ripe for innovative solutions.

References

1. *Nobel Lectures, Chemistry 1942-1962*, Elsevier Publishing Company, Amsterdam, 1964
2. Values provided by the Health Physics Society, North Carolina chapter, www.nchps.org

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