Instrumentation for Molecular Imaging in Preclinical Research

Micro-PET and Micro-SPECT

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Noninvasive imaging of molecular events and interactions in living small animal models has gained increasing importance in preclinical research. Two of the imaging modalities available for this research with potential for translation to the clinic are dedicated small animal positron emission tomography and single-photon emission computed tomography. This brief review introduces the fundamental principles behind these imaging technologies and instrumentation, and discusses the limitations in terms of their spatial resolution and sensitivity. In addition, it provides a perspective regarding the research and commercial development of these systems and presents examples of biological applications. Finally, it discusses the major challenges facing these technologies, advantages and limitations with respect to other technologies, and some future prospects.

Keywords: Small animal imaging; positron emission tomography; single-photon emission computed tomography; preclinical imaging

Animal models have been an integral part of biomedical research in the study of disease and physiologic processes (1). Tracer methods that rely on the introduction of a usually radioactively labeled molecule in very small concentrations were traditionally used, in combination with organ dissection and tissue counting (2). Alternatively, whole-body cryosection followed by digital autoradiography (3) can also provide anatomic images with exquisite spatial resolution ($\sim 50 \mu\text{m}$) correlated with quantitative measures of the tracer concentration. The drawback of these *ex vivo* methodologies is that only a single time point per animal can be sampled during an investigation. The lack of a method to study repeatedly the same animal model before, during, and after an intervention has been a significant limitation of these traditional investigative methods. Noninvasive positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging instrumentation that was originally developed for clinical applications had until recently inadequate spatial resolution for imaging small animals. Mice, the preferred mammalian models for genetic manipulation and biological research (4), are on the order of 2,500 times smaller than a human adult. A concomitant improvement in spatial resolution is required to achieve similar types of studies and results. That is a very large distance to be covered and the first attempts to bridge it were in the 1990s when the first dedicated imaging

systems being developed in different research laboratories made their appearance (5–7). Figure 1 illustrates the difference in spatial resolution between a typical clinical PET scan and different generations of dedicated small-animal PET scanners. The simulated image data are coronal cross-sections from a digital representation of a baby vervet monkey cortex, measuring 2.5 cm across.

PRINCIPLES OF PET AND SPECT

PET is based on the following principle. A typically cyclotron-produced (8) isotope decays with the emission of a positron (9). This positron travels a small distance in tissue depending on both the tissue density and the kinetic energy of the emitted positron. This energy is a property of the parent isotope, with a very short list of the most common ones illustrated in Table 1. After a series of collisions with atomic electrons from the tissues, during which the positron loses its energy and slows down, it annihilates with a nearby electron (Figure 2A [p. 510]) and produces two high-energy photons emitted in opposite directions. The simultaneous detection of these photons is the basis of PET imaging. The average distance that this positron travels from its origin is small and has biexponential shape (10). For the most commonly used positron emitters (F, C), this average distance is on the order of 0.5 mm or less. This positron range effect poses a lower limit to the spatial resolution with PET, achievable without the use of sophisticated mathematical models (11–14). Because every measurement with PET is composed by a large number of annihilations, some of the lost spatial resolution can be recovered with appropriate data treatment that estimates the expected location of origin (10, 11). At the point of annihilation, two photons with equal energy of 511 keV are emitted traveling at opposite directions (180° apart), conserving the total energy and momentum. Because of thermal vibrations in the tissues, there is an additional deviation from 180° , but this is a small effect for small animal systems that contributes to approximately 0.4 mm for a 20-cm-diameter tomograph (12). These photons travel with the speed of light toward the detectors positioned around the subject, where they interact getting absorbed and producing an electrical signal. The absorbing material of the detector is important, because it determines the interaction probability, which along with the geometric coverage area determines the system sensitivity (15). Furthermore, this material determines the accuracy with which one can measure the energy and time of the interaction. Typically, the materials used for PET are scintillators like bismuth germanate, lutetium oxy-orthosilicate, or gadolinium-orthosilicate (16), because of their high γ -ray stopping power and speed of signal conversion. The detector signals are further processed by specialized coincidence circuitry and, if the difference in the time of arrival of these photons is smaller than a predetermined value (typically ≤ 10 ns), then the two detectors define a line of response (Figure 2A). This is the geometric region between the two detectors in which the annihilation photons were detected. Many of these events

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Figure 1. Illustration of evolution of the volumetric spatial resolution of small animal positron emission tomography (PET) tomographs in comparison with a clinical system.

are stored and the data are subsequently fed into mathematical algorithms, which through a process called “image reconstruction” creates the spatial distribution of the concentration of the positron emitter in the field of view (FOV) (17). Consequently, the concentration of the labeled molecule in tissues can be determined in absolute units of $\mu\text{Ci/cc}$. The accuracy by which the location of interaction can be specified is closely related to the physical size of the detector elements and determines the size of the line of response. Consequently, the imaging system spatial resolution is also directly related to the physical size of the detector elements (14). Because the combined influence of other effects (photon noncolinearity and positron range) for the most common used isotopes is small, the dominant factor limiting the spatial resolution has been the construction of high-resolution detectors in continuous geometries (18, 19). Several research groups have developed high-resolution detectors (20–22), which enabled the development of high-resolution tomographic systems (6, 23–25). Although these early systems were developed as prototypes for in-house research in large research centers, their success and the newly developed need for marketed systems has led to the development of several commercial versions from manufacturers (26–28). These systems have increased the available useful imaging FOV, improved the total sensitivity, and more recently have combined multiple imaging modalities (PET or SPECT with CT) (29). Today, state-of-the-art PET imaging systems have the following features: volumetric spatial resolutions of 1.5 mm (3 μl) or better, an FOV adequate for single-position whole-body imaging of mice, and absolute peak sensitivity greater than 5% at the center of the FOV. This means that about 5% of the positrons emitted at the center are detected as valid events. The average sensitivity for all locations in the FOV is approximately half this value. Although this number might not sound too high, and there is still room for significant technological improvement, one needs to remember that the number of tracer molecules existing in each gram of tissue is proportional to Avogadro’s number. The overall sensitivity of PET translates to a capability to determine the concentration of molecular probes in the picomolar range *in vivo*. The overall spatial resolution can usually be improved during the image reconstruction process with the inclusion of physical models for the effects of detector blur, positron range, and other effects (30).

SPECT is also based on the tracer principle, but using a radioactive isotope emitting single photons rather than two photons from a positron annihilation. This single photon travels through tissues and needs to be detected on a position-sensitive detector, using similar detector technology as with PET (31). One of the most significant differences between PET and SPECT is that position detection of the photons in SPECT does not convey adequate information about its origin or the direction it

TABLE 1. COMMON ISOTOPES

	Isotope	Half-Life	Energy
PET	F-18	109.7 min	635 keV (max)
	C-11	20.3 min	970 keV (max)
	N-13	9.97 min	1.17 MeV (max)
	I-124	4.2 d	2.1 MeV (max)
SPECT	Tc-99m	6 h	140 keV
	I-125	60.2 d	30 keV
	I-123	13 h	159 keV

Definition of abbreviations: PET = positron emission tomography; SPECT = single-photon emission computed tomography.

was traveling, making the definition of a line of response impossible (Figure 2B [p. 510]). The direction of the traveling photon is determined by adding a lead collimator that acts as a γ -ray lens between the source and the detector. This collimator rejects most of the photons not traveling along certain directions and as a result the typical sensitivity of SPECT is several orders of magnitude lower than for PET systems. In clinical systems that require imaging of a large patient area, this collimator typically has parallel holes producing no magnification. Small-animal imaging, in contrast, requires higher spatial resolution for a much smaller object, and this is achieved by pinhole collimators (Figure 2B) (32). The trade-off between spatial resolution and FOV is natural in the setting of pinhole SPECT and very high spatial resolutions can be achieved (33). Furthermore, multiple pinholes can be used achieving a reasonable trade-off between spatial resolution, sensitivity, and FOV (34). Because the photon energies involved in SPECT are smaller than PET, and consequently the interaction efficiencies are higher, there is a much larger selection of available materials that can be used successfully for detector designs. Solid-state materials like CdTe have started appearing in commercial systems and have the promise to replace the traditional NaI scintillator (35). In a similar fashion as the PET scanners, small-animal-dedicated SPECT systems have appeared in recent years from commercial vendors. A list of vendors can be found at www.mi-central.org.

APPLICATIONS

The availability of small-animal PET systems with excellent temporal resolution, good sensitivity, and whole-body coverage has enabled imaging protocols that were not possible in the past. In addition to the more traditional imaging of xenograft tumor models, or other region of interest-specific protocols (36, 37), one can now perform first-pass angiography or look at whole-body pharmacokinetics (38). As an example, Figure 3 (p. 511) illustrates coronal sections from a mouse injected with a probe that is designed to enter hepatic cells where it becomes phosphorylated and trapped, depending on the levels of expression of the herpes simplex virus thymidine kinase gene (39). The *first panel* illustrates the flow of the probe from the tail vein injection, to the lungs followed by the systemic circulation, to the kidneys for clearance toward the bladder, while the liver retains the compound. The *lower panel* illustrates region of interest analysis of each of the organs and demonstrates the capabilities of the quantitative kinetic analysis. The *panel on the right* illustrates a volume-rendered combination of PET and CT of that image. More examples of applications can be found (36, 37).

DISCUSSION

Other imaging modalities discussed elsewhere in this issue, especially optical methodologies, have the advantage that the imaging

signal can be turned on or off depending of the presence of one or a combination of molecular events. The result is that optical imaging has inherently low background and can be very sensitive. Nuclide technologies rely on the kinetics of the molecular probe, especially on the clearance of nonspecifically bound compound. Optical signal, however, suffers severe attenuation and scattering as it travels through tissues, and modalities that depend on the transmission of light photons (including the near infrared part of the spectrum) are largely nonquantitative (in the strict sense as radionuclide modalities are). PET and SPECT photons are also not immune from attenuation and scattering, but these effects are many orders of magnitude less than for light photons and have much smaller dependence on the tissue characteristics. Corrections for these effects in radionuclide imaging are routinely implemented, and the resulting data can be accurately quantitated. Most important, the use of either of PET or SPECT in small-animal biological research has the advantage that essentially the same methodology can be used in human applications, thereby facilitating translation to the clinic. The notion that SPECT is unique in magnification imaging is not entirely true. PET images can be magnified with specialized detectors in the same fashion as SPECT, and this higher resolution imaging method could in principle be applied to human imaging (40).

Throughout the use of nuclide technologies, radiation dose to the animals is an issue that needs to be considered. Because of the higher spatial resolution requirement, the injected concentration of the labeled compound per gram of tissue is typically much higher. A typical ratio is 20 times or higher concentration in a rodent versus a human. Although for most protocols this dose is still below mass levels and does not violate the tracer principles, dosimetric considerations need to be taken into account (41). The design of more sensitive systems ameliorates this issue to some extent, but that requires new detectors with higher stopping power. Gating for cardiac or respiratory motion is relatively straightforward, but with unique challenges because of the high cardiac rate of mice (600 resting beats/min). In this case, too, higher sensitivity is important, because the short duration of the gates indicates that a very small number of counts will be detected. Other challenges are imaging protocols with animals under pathogen isolation, because many models have reduced function of the immune system, and temperature and anesthesia monitoring and control.

CONCLUSIONS

Small-animal PET and SPECT are enabling technologies available today for imaging research applications on rodents and other small-animal models. The technology continues its rapid evolution, and as it matures more commercial vendors will enter the field. The main advantages of nuclide technologies are concentrated in two areas: quantitative accuracy independent on location in the subject body, and direct translation possibility to human clinical setting.

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