Chapter 2
Imaging in Drug Development: Animal Models, Handling and Physiological Constraints

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Abstract In this chapter we delve into the primary components and operational issues that need to be considered in the setup and operation of a dedicated animal imaging facility with the use of PET, SPECT, CT, MR, and other platforms. The special conditions required for successful animal models, needs of the animals during imaging, and the avoidance of environmental and physiologic artifacts to the images are discussed.

2.1 Introduction

Preclinical molecular imaging research using animals typically focuses on measuring a metabolic process associated with a disease using PET, SPECT, or optical methods, often in conjunction with an anatomical imaging modality such as MR or CT (Phelps 2000). The purpose is to elucidate diagnostic or treatment options for a disease, then translate those findings into human applications. It is vital to understand whether the measurement being made is relevant, since handling conditions can alter the in vivo distribution of the imaging probe (Fueger et al. 2006).

With the advent of imaging systems suitable for work with mice, most preclinical research now utilizes mice or rats, which often provide very useful indications of what will happen in humans (Stout and Zaidi 2009). However there are situations where these animals have differences from humans that could be important. For example, rodents typically metabolize substances much more rapidly than humans,

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resulting in shorter circulating blood times, and if investigating the metabolism of a radiolabeled drug, the metabolites (including the radiotracer) may be cleared faster resulting in higher radioactive dose to bladder wall than might be estimated using other species. For preclinical imaging work, perhaps most important of all is that the biodistribution of imaging agents can be markedly altered based on how the animals are treated and how the data are both created and analyzed. Anesthesia, heating, and other factors alter physiological conditions (Fueger et al. 2006; Lee et al. 2005; Toyama et al. 2004), which in turn alter the images of metabolism obtained from PET scans by altering both distribution and kinetics of metabolism. There are often multiple choices for constructing PET and SPECT images (i.e., filtered back projection, ordered subset expectation maximization [OSEM], maximum a posteriori [MAP]), and the way images are analyzed can be quite subjective.

Characterization of how labeled imaging agents behave in vivo can be determined in several ways, including single imaging sessions, imaging the same animal multiple times, sacrificing animals at various time points for whole-body static (in situ) images, or individual tissue gamma counting for organ quantitation (percent of injected dose; %ID) or histologic/*en face* (liquid photographic emulsion) autoradiography (see Chap. 6 by Solon and Moyer). Whether optical, PET, SPECT, or MR methods are used, except in rare cases, anesthesia is essential to ensure the animals stay immobile during the imaging process. All anesthetic agents alter physiology, which in turn alters in vivo metabolism (Toyama et al. 2004). The question is what these effects upon the experiment at hand are and what can be done to control, minimize, measure, or remove those effects on the process under investigation.

This chapter will describe factors that can alter metabolism and measurements made in vivo. There are multiple ways to mitigate, measure, or control these factors, and the best method depends upon the specific experimental conditions. An example of the process of taking an imaging probe from idea to FDA approval through investigational new drug (IND) approval is presented as an example of how various preclinical investigation methods can be combined to take an imaging agent from an idea to clinical application and how one may utilize such agents in new novel drug and biologic discovery and development.

2.2 Animal Models

The choice of animal model to use depends on many factors, including availability of an appropriate model of the human disease, size with respect to the imaging system capabilities and resolution, overall operating cost, animal housing considerations, and perhaps whether the species of choice is well characterized for the type of experiment and data analysis required. In some cases, use of endangered species and animals commonly kept as pets is constrained by both regulatory and safety concerns. In some cases, drugs and biologics for treating certain threats (smallpox, anthrax, radiation, chemical warfare agents, or select agents (National Select Agent

Registry, CDC, http://www.selectagents.gov/select%20agents%20and%20toxins%20list.html)) have only animal models to demonstrate efficacy since testing in humans would be unethical (see the Animal Rule: 21 CFR 314.600 for drugs; 21 CFR 601.90 for biological products; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078923.pdf).

Larger species, such as dogs, primates, or pigs, have advantages with respect to how well they lend themselves to observation and measurements of small structures. The limited resolution of imaging systems means that a larger object may be the only way to measure certain structures, especially in imaging substructures of the brain. Large animals have bigger blood pools that may be needed for rapid and frequent blood time-activity sampling and metabolite analysis. Surgical interventions are easier and most veterinarians or medical doctors are able to work easily in larger animals.

The drawback to larger animals includes high cost, difficulty in handling, and that they often can be imaged only using human imaging systems. There may be regulatory barriers to imaging animals and humans in the same system or difficulty with availability of scanner time (clinical use over animal use) and radioisotope selection. Many primates are endangered, and even with breeding colonies there are often high costs and safety issues that preclude using primates. Perhaps the biggest limitation is the small number of studies, usually just one or two, that can be done per day compared to smaller species using miniaturized PET and SPECT systems. Furthermore, larger animals require expensive and large caging systems and require careful handling for physical safety, since there may be biological pathogens and parasites that could be transferred to humans, such as hepatitis and tuberculosis.

There are times when the only justifiable species to use may be primates. One example is the study of Parkinson's disease, where biochemical, behavioral, and in vivo metabolic information can be obtained. The larger size of primates enables visualization of the caudate and putamen brain structures, which in turn allows for earlier and more accurate detection of the disease. Further, primates replicate the human disease very well using a neurotoxin-induction model that does not affect rodents (Leenders et al. 1988).

Small animals such as mice and rats have a number of advantages. The entire animal can often be imaged all at once, making it far easier and more accurate to measure biodistribution of probes and radiation dosimetry. Smaller animals are less expensive and easier to handle, and large numbers can be housed together and imaged in a short time. The smaller sizes also afford less scatter, attenuation, and less radioactivity needed for imaging, which also reduces personnel exposure. Mice in particular are available with a wide range of genetic knock-in and knockout genes, thus can be used to look at many different genetic conditions. The availability of immune-compromised SCID and nude mice and rats enables oncology research using human tumor lines, which means that experimental results should ideally translate directly to human use.

However, rodents are not always the ideal option because surgical interventions, blood sampling, and injections are not easy. The small size means that in general only organ-level measurements are possible using most methodologies, and long

scan times are likely necessary to obtain high-resolution MR images. In some cases, rodents are not well matched to humans for certain diseases. One example is the lack of a gallbladder in rats making gastrointestinal imaging and studies where biliary clearance is important difficult to investigate and interpret.

2.3 Animal Handling

From the time animals are received from a vendor until completion of the project, there are many steps where environmental conditions must be carefully considered. A well-designed imaging center or neighboring coordinated facilities are necessary to conduct the necessary procedures to create the animal models (Fig. 2.1). For imaging work, heating, anesthesia, pathogen control, positioning, and post-imaging disposal are necessary to acquire useful data in a safe and effective manner. Even before work begins, animals must be acclimatized to their surroundings to reduce the stress of transport (Conour et al. 2006; Jennifer Obernier and Baldwin 2006).

For genetic and oncology work, there are biosafety concerns for viral vectors, human tumor cell lines, and potentially carcinogenic or biohazardous chemotherapy agents. An appropriately furnished location is essential to safely work with these agents, monitor tumors, and conduct any surgical interventions. Local regulations vary widely, but the general requirements can be considered to be a barrier facility with proper ventilation, biohazardous capabilities, and perhaps a surgical area with anesthesia and heating support.

Depending on local requirements, it may be necessary to quarantine or isolate animals based on their health status, immune condition, or due to the use of

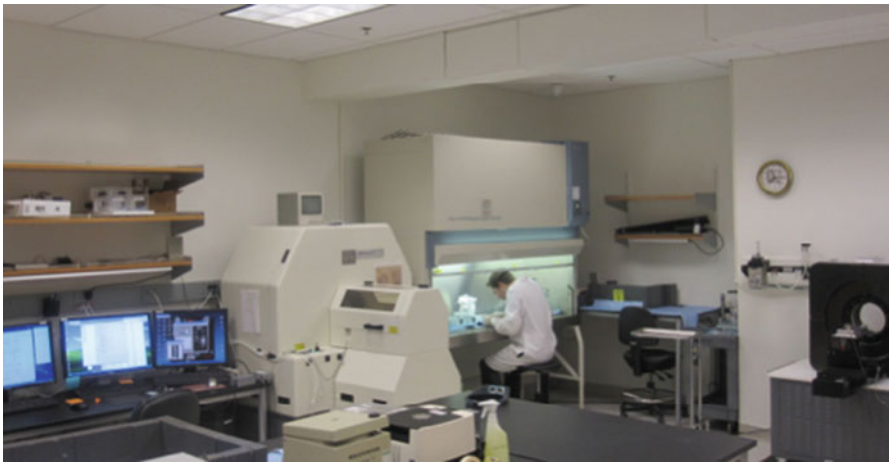

Fig. 2.1 Good ergonomic design puts everything in close proximity where needed and uses space for radiation safety where possible. The biosafety cabinet is located between the PET and CT systems, with the computers located to one side to avoid radiation exposure

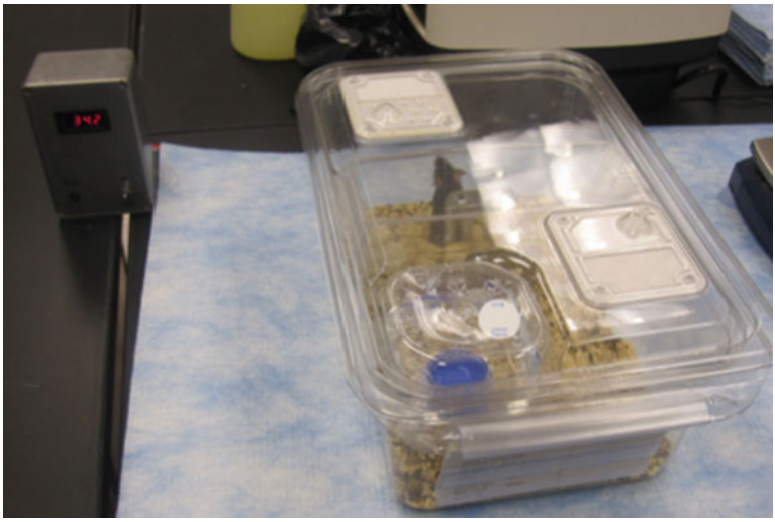

Fig. 2.2 Heating plates are located throughout the imaging center, positioned wherever animals are located. Preheating animals ensures minimal brown fat activity and a stable reproducible environment essential for reproducible metabolic data

infectious or select agents (see Chaps. 9 (Golding and Zeitseva) and 10 (Keith et al.) on imaging infections with optical probes and under regulated biosafety level (BSL-3/4) containment, resp). Quarantine may be due to disease or parasites or might be due to the use of neurotoxins or other biohazardous agents that may be excreted and pose a handling risk. The space requirements for this can pose a serious problem and might prevent experiments if no suitable locations are available. One option frequently used is the use of disposable microisolator cages (Fig. 2.2), where each animal is in an enclosed space without exposure to adjacent cages. This requires the use of individually ventilated cage racks (IVC) and cage changing using a biosafety cabinet and proper barrier techniques. One caveat is that IVC housing systems can create or exacerbate cold stress in animals, which may have deleterious or unexpected consequences upon translational research.

For imaging work, nearly all studies are done with animals under anesthesia. This is necessary to acquire sufficient data over a period of time to create a useful image where mobility affects positional information while it is being acquired by an imaging platform. Animals might be imaged dynamically from the time of injection or may be allowed a period of time for uptake and nonspecific agent clearance prior to a brief static imaging session. For dynamic work, typically 1 h is sufficient for mice and F-18 (positron isotope; ~2 h physical half-life)-labeled probes; however, this is highly dependent on the imaging agent, its biologic half-life in the species of interest, and its physical half-life. For experiments requiring longer times to observe specific biomarker signals, often animals will be injected with isotope having 12–72 h half-lives and the animals imaged multiple times over several days. For static imaging work, there may be relatively large numbers of animals being imaged

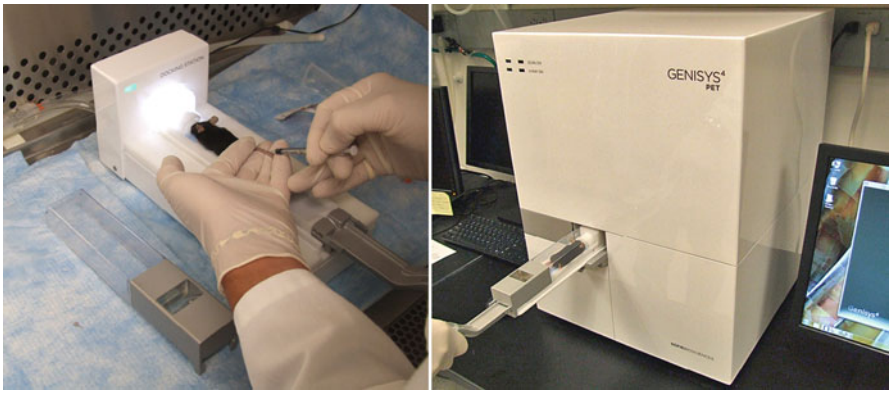

Fig. 2.3 This system has no lines to connect for the chamber, a simple plug and scan configuration. The system has both X-ray and photographic imaging components; thus, anatomical information is provided and CT imaging may not be necessary. The system has video monitoring of animal that provides a readout of the respiration rate, thus providing information to adjust anesthesia accordingly

together. These animals might be injected with short-lived isotopes such as F-18 and imaged multiple times over the course of several weeks using repeated injections.

Ideally the imaging systems will have proper physiological support systems for establishing and maintaining a reproducible metabolic state. To date, few systems provide these features, despite the importance of stable physiology for measuring changes in metabolism. Several equipment vendors have started offering heating and anesthesia options, along with imaging chambers; however, most are still unfortunately third-party additions to the system. Recently a new system has become available that integrates anesthesia, heating, chambers, and a prep station for comprehensive PET imaging for mice (Fig. 2.3).

To keep track of all the animals, data files, billing, and usage information, a database is essential (Fig. 2.4). The archival system, animal identification, and session information can be part of the database, making it simple and easy to track information for each experiment. Password protection and access strategies can be put into place to enable restricted and flexible access to both the database information and image data. The example shown in Fig. 2.4 is used to create session information, billing reports, usage reports for grants, oversight committees, and other needs, along with features for archival and retrieving of data.

To aid in accurate data analysis and animal safety, reproducibility of positioning is highly desired. This can be accomplished through the use of imaging chambers designed to deliver anesthesia, heating, and reproducible positioning (Suckow et al. 2009). Properly designed chambers can provide a pathogen-free environment necessary for working with immune-compromised rodents typically used for oncology research (Fig. 2.5). To facilitate high throughput of animals in a cost-effective manner, the anesthesia systems ideally should be simple and easy to use without requiring adjustable flow meters.

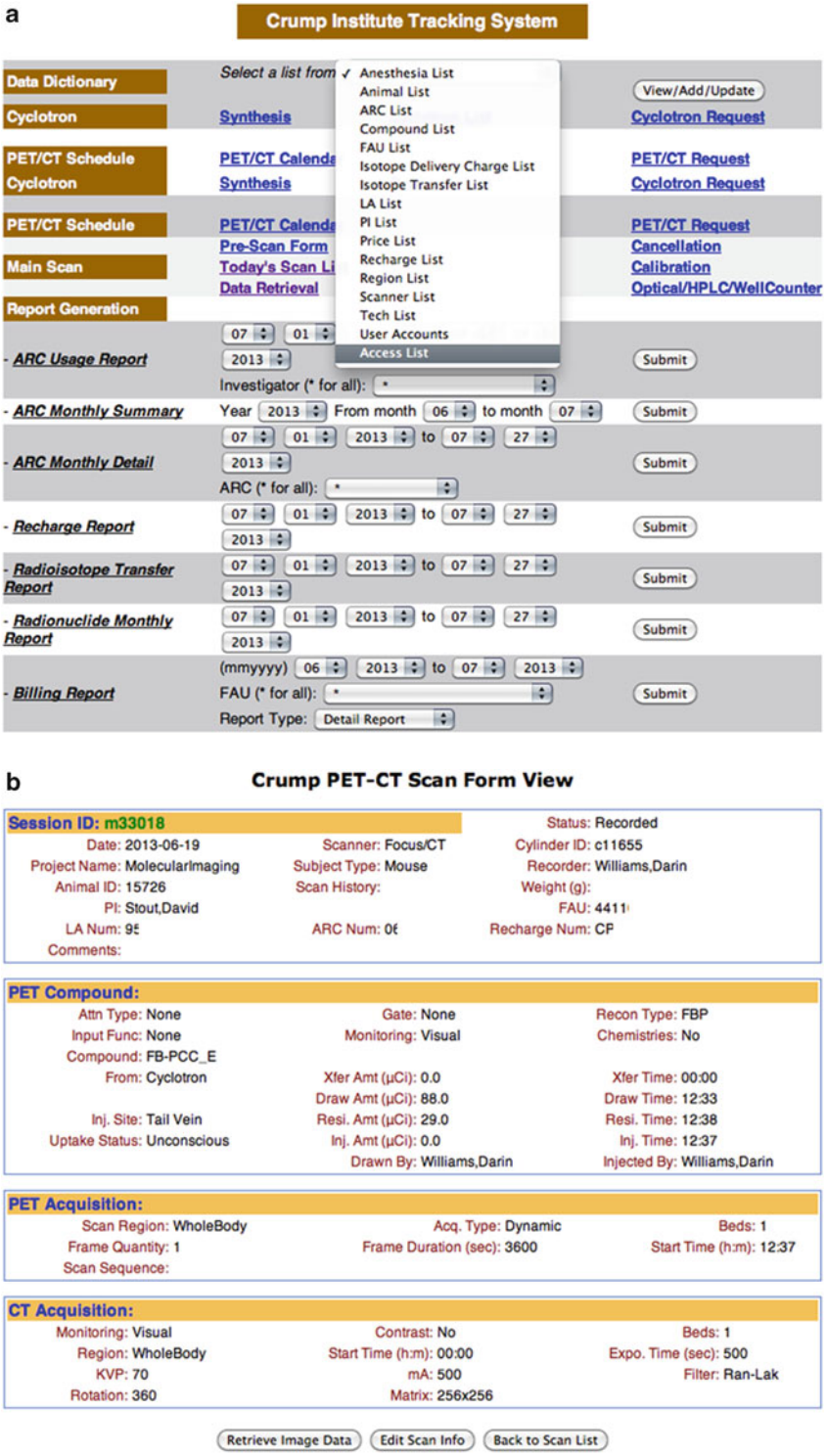

Fig. 2. 4 An easy to use image archiving strategy is essential. Database must include all relevant data related to the experiment, both for investigator's subsequent image analysis and for reports generated for various regulatory agencies and grant reviews

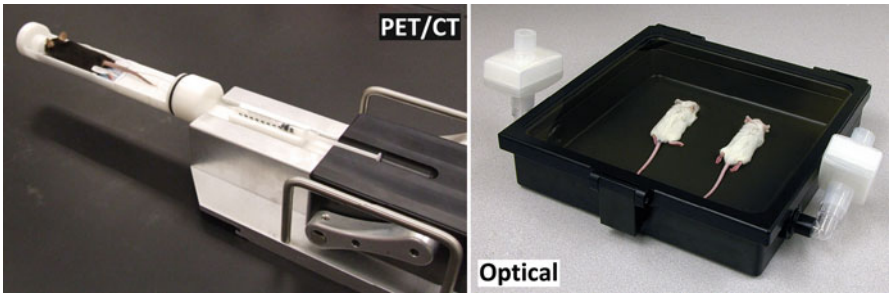

Fig. 2.5 The microPET-CT imaging chamber provides reproducible positioning, constant gas anesthesia, multimodality imaging capability (PET, CT, MR), barrier for immunocompromised mice and rats, and temperature control. The optical chamber provides gas anesthesia and barrier conditions

2.4 PET/CT

The logistics of imaging multiple animals in a short period of time requires good ergonomic design of the workspace (Stout et al. 2005). Our center primarily images immune-compromised animals; thus, we conduct all of our animal preparation work in biosafety cabinets. To avoid mistakes, use of two gas anesthesia boxes is recommended to separate injected from non-injected animals (Fig. 2.6). Heating should be provided for cages, induction boxes, work areas, chambers, and recovery areas to ensure mice are properly warmed to facilitate good blood flow and stable enzymatic activity. Without heating, animals will rapidly become hypothermic under anesthesia, and this will alter physiology and any metabolic measurements. This can result in poor blood flow to peripheral and subcutaneous tumors and will highly activate brown fat in the neck region (Baba et al. 2007) (Fig. 2.7). Mice thermoregulate body temperature via tail blood flow, so the ability to inject and deliver probes via tail vein injections depends on both tail and body temperature.

The injection of an imaging agent can be quite challenging. The small volume, typically 50–200 μl, small size of the mouse, and high energy of PET radiation mean that use of a shielded syringe is not feasible or practical. The better option to reduce the hand dose of the animal handler is to practice and become proficient in quickly injecting mice using an unshielded syringe. Most injections are made into the tail vein of animals. However, intraperitoneal or retro-orbital injections are sometimes possible depending on the experimental conditions.

One major complication with injecting in the tail is the unknown amount of radioactivity which extravagates and is left in the tail. Usually the injection location is not within the field of view of the imaging system, and the amount left behind is unknown, is highly variable, and can be quite significant. This complication can lead to highly inaccurate estimates of the total injected dose for subsequent

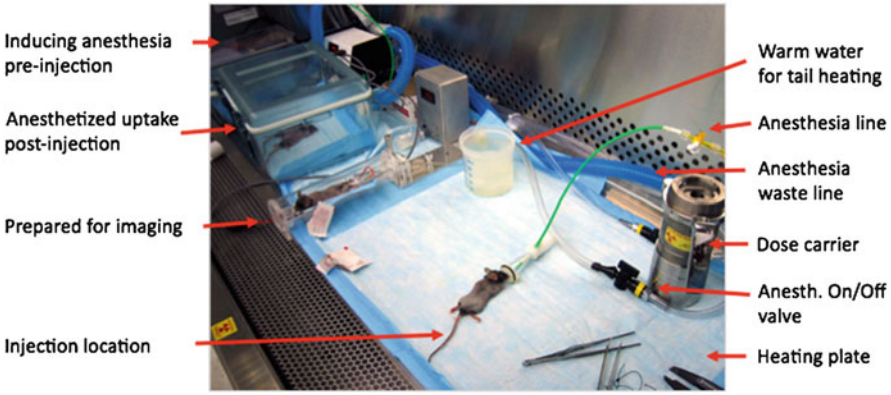

Fig. 2.6 Usually several mice are undergoing injection, uptake, and preparation for imaging at the same time. With the demands of anesthesia, injection, chamber assembly PET scanning, CT scanning, and recovery happening in each 12 min block of time, the process needs to be straightforward and everything within easy reach. Set-it and forget-it arrangements for heating and anesthesia are essential

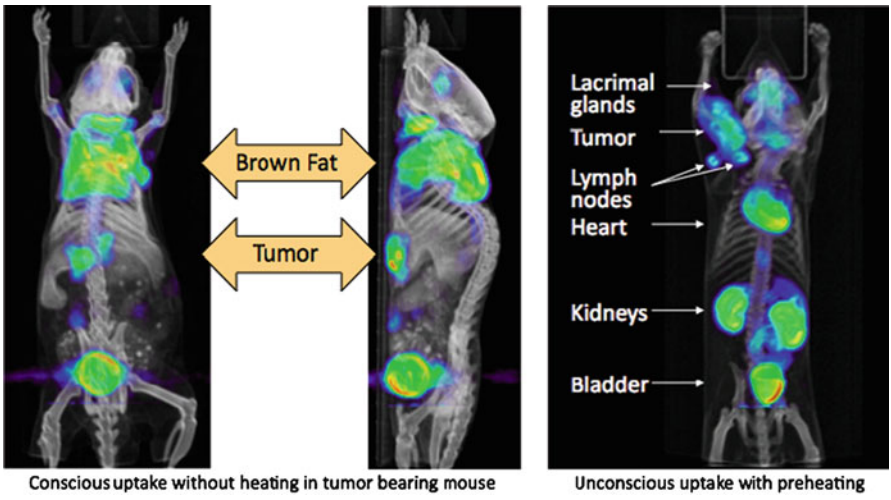

Fig. 2.7 Cold animals compensate by activating brown fat, a highly metabolically active tissue that can mask nearby FDG signals. Proper preheating can eliminate this signal

quantitative analysis. Practice and developing good injection expertise or the use of a catheter to inject can reduce the residual activity. If accurate injection activity is needed, it is best either to measure the tail activity or to quantify based only on what is present in the body, excluding the tail activity since it was not bioavailable for metabolic determinations.

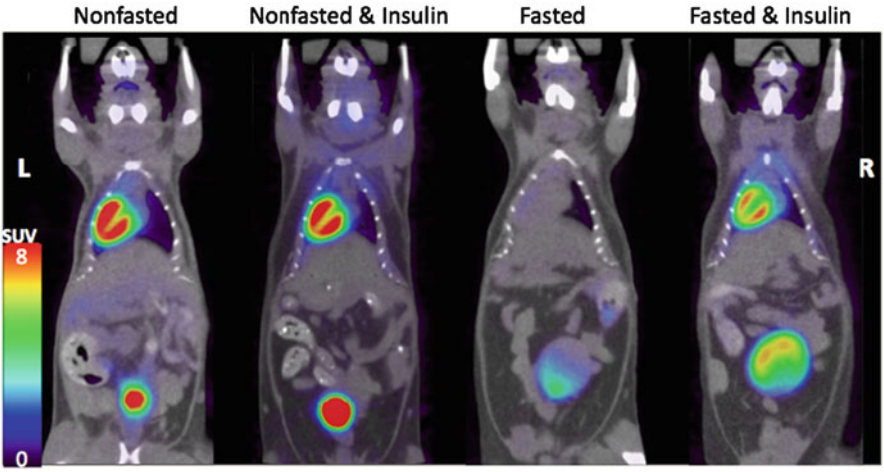

Fig. 2.8 Endogenous glucose competes with FDG for tissue uptake. In the heart, fatty acid versus glucose utilization plays a big role in myocardial tracer uptake

How animals are injected, treated, and handled can dramatically alter the uptake pattern of imaging probes. An example of this is the ability of heart myocardium muscle to switch from glucose to fatty acids as an energy source (Fig. 2.8). If mice are fasted, the heart uptake of FDG can be essentially switched off (Kreissl et al. 2011). This might be unwanted for cardiologists but could be ideal for looking at signals in the lungs. Another example for oncologists is the uptake of imaging probes in peripheral subcutaneous tumors. If animals are hypothermic, blood supply is constricted to preserve core body temperature, so probe uptake may be dramatically reduced due to temperature rather than intervention (Fueger et al. 2006). Often tumors are placed on the upper flank to avoid bladder signals. However, with FDG there can be extremely high brown fat uptake in the neck region that may interfere with tumor measurements (Fig. 2.7). Many imaging probes cross through cellular membranes by active transport, which is subject to competition by endogenous compounds, such as glucose or amino acids. Often probes are specifically retained by phosphorylation by enzymes, which are known to be linked to temperature and circadian rhythm (Jilge 2004). Rodents thermoregulate their body temperature based on blood flow in the tail, which may alter tail injection clearance and blood pressure and flow.

2.5 Biodistribution and Radiation Dosimetry

When developing a new imaging agent, the most common experiment is to look at where the radioactivity goes over time. These biodistribution studies can be relatively short, 1–2 h, or may require multiple imaging sessions over time, based on the pharmacokinetics of the labeled probe. Rodents can usually be imaged safely for

1–2 h; however, longer times are possible if subcutaneous saline is given to help keep the animals hydrated. Many small molecules will clear fairly rapidly in rodents within 1 h, either by renal kidney excretion or through hepatobiliary excretion via the gallbladder from liver through the gastrointestinal tract. Most F-18-labeled probes will rapidly accumulate in the bladder. It is important to understand the elimination process, in order to design the targeted uptake region to avoid these structures. For example, tumors are best placed in the upper flank or shoulder region, rather than anywhere near the bladder. Labeled peptides or antibodies typically require many hours or even days for specific targeting and clearance of nonspecific signal; thus, these experiments will require imaging over the course of several days (Kenanova et al. 2005).

Biodistribution data is typically expressed as the amount of injected dose in each organ over time. This requires knowing the amount that was injected, the amount in each organ over multiple time points, and the organ weight (i.e., percent injected dose, %ID, and %ID/gm over time). As mentioned before, knowing how much was injected is not as simple as measuring what was in the syringe, but rather how much activity was available to the body over time, disregarding anything remaining at the injection site. When dynamic imaging is started from the time of injection, a simple way to check on the injection quality is to look at the whole-body total activity over the course of the imaging session. Since the activity is decay corrected for the isotope half-life by the image reconstruction software, the total activity seen in the image over time should rapidly reach a peak and stay constant over time. If the activity rises, then there is leaching in of activity from outside the field of view (typically from the tail injection site), as the animal is not capable of generating radioactivity on its own.

With the knowledge of the amount of radioactivity in each organ over time, determining radiation dosimetry is fairly straightforward. By removing the isotope decay correction, the number of disintegrations over the course of imaging can be determined. Presuming that changes in biodistribution are fairly complete by the end of the imaging time, then one can safely assume that the remainder of the radioactivity will stay in the same location. By taking the last biodistribution time point and assuming no change in location, one can compute the number of disintegrations for the next five or so half-lives, which will account for over 95 % of any dose. Integrating the total dose over time, the dose per organ can be determined, and the dose per injected activity amount can be established. The term used for the number of disintegrations in any given organ is called the "residence time," which is a function of both the biological and physical half-lives of the labeled probe. Using Olinda, the only FDA-approved dosimetry software package, the amount of dose delivered to each organ can be determined (Stabin et al. 2005). From this data, the maximum safe injectable dose for humans can be determined and used to obtain approval for first use in human studies with new imaging probes.

Animals provide a reasonably accurate estimation of human radiation dosimetry (Seltzer et al. 2004). Although historically primates or larger animals have been used, it turns out mice provide reasonable and slightly conservative estimates. An advantage to mice is that since the entire animal can be observed at once, better temporal sampling is acquired for all organs at the same time.

2.6 Metabolic Profile and Toxicology

It is vital to keep in mind that nuclear medicine images, whether SPECT or PET, provide information only about the location of the isotope decay. Excluding positron range, the images show where the isotope was located, but not what atom it was attached to at the time. A decayed and recorded photon event in an imaging system could have come from either free isotope, a metabolite, or from the intact labeled imaging probe. Without knowing the metabolic profile of the imaging agent, there is no way to know what the image signal actually represents. For this reason, it is imperative that the metabolic fate of the injected probe be carefully studied in vivo before any claims are made about the performance of the imaging agent.

Characterization of a labeled probe is typically accomplished by taking blood samples over time and examination using high-pressure (or performance) liquid chromatography (HPLC). Imaging probes can remain unchanged in vivo or may be phosphorylated or metabolized into a different molecule. Retention times of known standards measured via UV absorption versus the retention times of the radioactive peaks indicate the metabolic fate in vivo. Understanding what the images are showing is essential to understanding the experimental results.

When the time comes to move an imaging probe into human application, it is necessary to ensure that there are no toxicological effects of the imaging probe resulting from normal metabolism. Toxicology testing is accomplished by injecting generally 100× the normal expected mass amount of the imaging agent into animals and observing the physiology for any changes. It is not necessary to inject radiolabeled probe, so this work can be done using the unlabeled substance itself. Given that most PET imaging agents are used in picomolar or nanomolar concentrations, toxicology testing is often accomplished using only 1–2 μg of the compound. This tiny amount of material is difficult if not impossible to detect by any means other than having it labeled with radioactivity. To date, no probe examined by or known by the author has elicited any physiologically recorded response or safety issues following injection at the concentrations expected for routine imaging use.

Since toxicology testing can be very rigorous and expensive, it is worth making the distinction between testing required for FDA approval of a new agent and what is required for preliminary first use in human (FIH) studies. For initial evaluation, testing in rats is ideal as the species is sufficiently large to acquire blood samples prior to injection, immediately after injection and at 2 h postinjection. These blood samples are examined for any changes, and it is important to separate changes normally found with anesthesia from those that might possibly occur due to the injected agent. The rats are examined frequently over several hours to look for any physiological changes in temperature and heart and respiratory rates. This preliminary data is sufficient to show lack of any effect and to obtain initial approval for first use in humans for compounds already approved by the FDA.

When the time comes to seek FDA approval under the investigational new drug application (IND) for more routine use in humans or for a new molecule not previously approved, more stringent toxicology testing conducted under regulatory mandated good laboratory practices (GLP) is required. The guidelines for these

procedures are available through the FDA website (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/default.htm). These requirements are similar to the cGMP requirements for producing radiolabeled human use probes and describe the need for careful monitoring and validation of each step in the process. An independent auditor must review the data to make sure everything is conducted according to plan and that any deviations are properly documented. Toxicology testing at this level is often quite expensive and until recently has required evaluation in two separate species. As will be explained below (and Chap. 13 of this volume), another option is to develop in-house services as opposed to the more expensive contract research companies.

2.7 Radiochemistry

It is worth noting the distinction between the conditions now required by the FDA for imaging probe production for human use versus nonhuman use. Since December 2011, all production of labeled imaging agents used in humans must follow cGMP requirements laid out by the FDA (Norenberg et al. 2011). Production of preclinical research probes generally falls under the US Pharmacopeia regulations, though this is not clearly defined for preclinical work intended for eventual human use authorization. Given the high cost differential between various production options, it is important to know ahead of time what conditions are required for regulatory approvals.

2.8 From Idea to IND: The Story of FAC

At the author's institution, a decision was made to develop a new PET imaging probe targeting immune system cancers for diagnostic purposes. One of the strengths of an academic institution is the availability of a diversity of expertise from multiple fields. A group of people was assembled and given a deadline of several months to identify candidates for looking at upregulated receptors in the activated immune system. We wanted to specifically look at only what was activated in a disease state, not in normal metabolic function. Using various tests, including microarray gene mapping, literature knowledge, and various in vitro tests, we arrived at a candidate molecule, called 2′-deoxy-2′-[18F]-D-arabinofuranosylcytosine (D-FAC). This gemcitabine analog is a specific substrate for deoxycytidine kinase (dCK) receptors, which are upregulated in activated lymphoid organs (Radu et al. 2008; Laing et al. 2009). The imposition of a deadline was useful incentive to move quickly, as was the presence of a leader (Dr. Michael Phelps) who kept everyone focused on meeting the goal in a time-efficient manner.

With the knowledge that there were several stereoisomers of the FAC molecule, we examined each of them with in vitro cell and in vivo preclinical studies to decide

the best candidates for subsequent human work. We examined three of the isomers in depth, including metabolic profiling and in vivo biodistribution and toxicology testing. We were able to synthesize the probes, investigate in mouse models and begin investigations in humans within 9 months, a staggering feat compared to historical trends of 10 years or more. For the second isomer, we did this in only 6 months. With further investigations using human subjects, we moved on to filing with the Food and Drug Administration (FDA) via the investigational new drug (IND) process, obtaining approval 30 days after submission. The third isomer was also submitted, in case it turns out that this isomer works better in humans compared to rodent results.

To accomplish this effort in such a short time, the right people, skills, and equipment were needed. Our group has a long history of developing new PET imaging agents, along with careful characterization of the biological fate in vivo and going through the approval process. For preclinical work, we had already put into place expedited approval processes, imaging equipment and procedures, and developed biodistribution and dosimetry services. We moved quickly by knowing how to make the regulatory system work in an expedited manner and from having the tools and techniques honed that would enable us to quickly acquire, analyze, and move forward with additional experiments.

One reason it has been fairly straightforward to obtain FDA approval is that PET probes are not a drug intended to treat any disease but rather an imaging agent used at only one dosage. For this reason, and since PET agents are used in vanishingly small concentrations (often nano- or picomolar), we sought approval using only one species (rat) and one dosage for toxicology. Simplifying the submission and providing only the relevant required testing results meant that the approval process was faster and much more economical. Estimates are that we obtained approval at less than 10 % of the usual costs for new IND filings for therapeutic indications.

The key to successful imaging probe development is an effective and efficient preclinical imaging infrastructure together with good radiochemistry and clinical support. These three often separate groups must work together to make and evaluate imaging probes and then move them through the regulatory process. We added our campus veterinary group to this triad, which helped establish good laboratory practices (GLP) for the toxicology testing program that would have been too difficult and expensive to pursue without their help. With the establishment of GLP toxicology testing, together with the approvals for animal work established through the preclinical imaging center, we now offer this service campus-wide and on a contract basis to outside parties.

Several services were added to the preclinical imaging center, namely, autoradiography, biodistribution, radiation dosimetry, and toxicology testing. These were added because the complexity, expertise required, and coordinated nature of conducting the studies would have been extremely difficult for any one faculty member to manage. Often these services are used intensely, but infrequently, so the knowledge and skills can be lost as students or postdocs rotate through labs or staffing changes occur.

Autoradiography support was added by purchasing a microtome for tissue sectioning and a whole-body slicing system. The whole-body cryotome was very useful in observing where the labeled probe went at various times with resolution down to the cellular level (Stout and Pastuskovas 2011). This was particularly important for FAC, as the gut uptake observed in the PET images was localized to the intestinal villi due to blood transport and receptor binding rather than transit of hepatobiliary excretion from the gallbladder into the GI tract. Although not used frequently, autoradiography was a crucial step to take in the process of identifying exactly what was being observed in the PET images. Autoradiography can provide detailed radioactivity distributions, down to about 25 μm, which is far greater resolution than nuclear medicine-based approaches (see Chap. 6 for more details on autoradiography applications). The reader is encouraged to read Chap. 6 (Solon and Moyer) for a full treatise on autoradiography.

Biodistribution kinetic information is readily obtained from dynamic PET or SPECT imaging by experienced personnel. The process of creating the optimal set of images to capture the changing distribution patterns takes time and skill, both with image reconstruction and image analysis. From this information, estimates of translating the animal biodistribution kinetic data to human radiation dosimetry can be measured. Olinda, the successor to MIRDOSE, is the only FDA-approved dosimetry program, which is used to determine the maximum safe allowable radiolabeled imaging agent dose for use with humans (Stabin et al. 2005). Fortunately mice usually provide a conservative estimate of a safe injectable radiation dose, since the limiting organ for many imaging agents is typically the bladder wall where renal elimination and persistence of a high concentration of radiolabel is most often evident. Mice often metabolize and eliminate into urine the radiolabel from imaging agents much faster than humans, so the maximum safe injection values we determine are often lower than what is later determined from human studies.

For toxicology, we took a two-step approach: simple tests for previously FDA-approved molecules via the Radioactive Drug Research Committee[1] (RDRC) approval and GLP level testing for IND filing with the FDA. To validate that there were no measurable changes in heart rate, respiration, temperature, or blood chemistry, we inject 100× the expected dose into five rats and monitored them for 1 h, followed by necropsy reports at 2 weeks. The amount of injected agent was 1.2–1.6 μg. At this low a concentration, it is not surprising that we saw no changes in physiological parameters. We did see some drift in the blood chemistry measurements over time, which we confirmed using saline injections in control animals to be related to anesthesia rather than any effect from the injected agent. These preliminary toxicology tests, together with biodistribution and dosimetry measurements in mice, were sufficient to satisfy RDRC requirements for first use in humans, for up to 30 patients.

An interesting and important note is that the probe that we found worked best in mice was not the one that works best in humans, at least not so far in our limited investigations.

[1] http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Oncology/ucm196481.htm.

This is one reason that it is important to pursue multiple imaging isomers and to begin work in humans as rapidly as possible. Considerable time, money, and resources can be spent in preclinical models which might not replicate well in humans. By moving drug development rapidly into human use, we can better know where to devote our time and effort for other clinical and preclinical research. A similar finding was noted by the group at UC Davis (Gagnon et al. 2009), where the predictions based on in vitro testing did not lead to the best candidate in vivo. Since they evaluated a range of different compounds, they were able to identify ones that were most suitable to move forward into human testing.

Once the ideal candidate was determined, the next step was to assemble the tests required for IND filing. The major requirement was GLP level toxicology testing. Where we could have pursued one agent together with one control group, we chose instead to evaluate three agents at once. This saved having to test two additional control groups, thus by pursuing all three agents at the same time; we spent 33 % less for the testing of four groups instead of six. Conducting the testing in-house, even with invasive surgeries to directly measure blood pressure, we spent ~$160K for four groups, both sexes, one species. We were prepared with animal use approvals to investigate a second species, but ultimately we were not required to do so.

To accomplish these tasks, instead of obtaining regulatory approvals for a specific investigator, we decided instead to create an animal use protocol for the imaging center which included all the necessary testing steps. Investigators can readily pursue their own research under their own approvals, but now they can also ask for these tests to be done and do not need to devote their lab's personnel, time, and resources to carrying out these tasks. These tests are often infrequent, so having central staff trained and able to consistently carry out the tasks save having to train new students, staff, or postdocs in various labs who may turn over between tests. All the necessary steps can be put into one protocol and maintained ready for any group to use as needed.

In addition to the preclinical work to evaluate the dosimetry and toxicology, the IND filing required a description of the mechanism of action (MOA), metabolic fate, and a detailed description of the synthesis conditions (IND CMC requirement). For use in humans, a plan of action such as a clinical protocol and expected outcomes of that trial are required, along with any previous information about use in humans (i.e., outside of FDA jurisdiction).

The regulatory environment is a considerable hurdle and can at times become a substantial roadblock to research. Working together with these oversight agencies, a fast-track system can be established for adding new imaging agents to approved protocols. When one considers that everything done in an imaging experiment is essentially identical to previous imaging work, other than what is within the injection syringe, adding a new agent that is usually in the nanomolar range should be a simple authorization that does not require a full committee review of each agent. For a more detailed view of the regulatory landscape for imaging in general, please see Chap. 13 on Regulatory Issue with Imaging.

2.9 Summary

Successful and accurate preclinical data acquisition in support of regulatory authorization for a novel imaging agent requires a well-designed and well-integrated imaging center that supports all the logistical elements of the process. Attention to detail is critical, as a seemingly minor problem may invalidate the entire filing process, requiring additional work. Good design for flow of people; animals; radiation usage, including human subject dosimetry estimation; and data management can help to ensure that standardized procedures are followed, appropriate data are collected, and optimal physiological conditions for metabolic imaging are followed.

Once data has been acquired, processing of images into useful metabolic information requires in-depth knowledge of the imaging systems and image reconstruction and a clear understanding of the physiology of the selected animal models. For FDA filings to use a new imaging probe in humans, a careful understanding of the regulatory requirements and documentation of the entire experimental process is essential. The scope of knowledge required will almost certainly require a team effort of people with a variety of skills, including physics, biology, radiochemistry, statistics, dosimetry, and regulatory compliance. While managing to meet all these requirements is daunting, once the process has been created and documented, subsequent work can become fairly routine.

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