DESIGN AND SETTING UP OF THE UNIT OF MOLECULAR IMAGING OF LARGE ANIMALS AT THE NATIONAL CENTRE FOR CARDIOVASCULAR RESEARCH

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Abstract

Molecular imaging techniques have become important tools for the clinical diagnosis of several diseases. These techniques are today in a highly mature state in the clinical field and are now being rapidly developed for use in biomedical (preclinical) research. Among currently available techniques are those allowing acquisition of high resolution anatomical images (CT, MRI), while others offer high sensitivity physiological/molecular imaging (PET, SPECT, optical). However, used separately each technique offers limited information, and therefore the emphasis of imaging applications for research is on multimodal imaging, wherein images from different techniques are combined to yield an image of high resolution and sensitivity.

Although the use of radioactive isotopes in biomedical research is declining overall, their use in molecular imaging techniques is increasing. Currently, the most developed molecular imaging technique in research, and the most significant from the perspective of radiation protection (RP), is the microPET, combined with anatomical imaging, mainly by CT. In order to set up this technique (or others, such as SPECT) in a biomedical research centre, the RP requirements associated with the handling of high energy gamma sources (PET) and X rays (CT) must be met (equipment, shielding, dosimetry, waste management, training, etc.). These measures also need to be evaluated and adjusted to meet the specific requirements of research centres in terms of biosafety, animal health and welfare, etc. This situation thus complicates RP in this kind of facility.

The aim of this study is to briefly describe the most important imaging techniques and their application in biomedical research, and to present an example of the setting up of a unit or laboratory specialized in these techniques in centres dedicated to pure biomedical research (not associated with a healthcare centre).

This study has been conducted by specialists from the RP and molecular imaging fields.

KEY WORDS: molecular imaging techniques, biomedical research, non-sealed sources.

1. INTRODUCTION

Biomedical research addresses increasingly complex problems related to the biochemical processes that occur in living organisms. As in clinical research, medical imaging techniques are excellent tools to study these processes. A molecular image can be defined as a visual representation, characterization, and quantification of biological processes at the cellular and subcellular levels within living organisms without disturbing the system under study.
Different Molecular Imaging techniques are combined in this single discipline with a common aim: to change the way in which biological research is carried out. Techniques such as, among others, PET, single photon emission computed tomography (SPECT), digital autoradiography, magnetic resonance (MR), MR with spectroscopy, bioluminescence, fluorescence, and echography, are under constant development. Lately, considerable effort has been directed towards the development of these non-invasive high-resolution imaging techniques for their use in large animals.
The aim of this work is to describe the different aspects to be take in account while designing and setting up a molecular imaging unit others than the image process itself, like the special size

animal models, dealing with such models implies higher dose injection, larger volume waste, and hardest handling specimens.

Positron Emission Tomography (PET)

By means of PET the metabolic route can be visualized that a given molecule follows after its incorporation into the organism, generally by means of intravenous administration of a radiolabelled drug. Various biological molecules are labelled with positron-emitting isotopes and follow their normal metabolic route, moving to the sites where they are metabolized[1]. Throughout their course, and also from the sites were they are stored and eliminated, they emit a radioactive signal that can be detected from the outside. Detection is done by means of a positron camera or a PET camera. At present, the basic radiolabelled drug used in this technique is fluorodeoxyglucose (FDG), a glucose analogue labelled with Fluorine-18. This tracer is relatively unspecific and, although it allows to locate and to study in detail, the uptake of the molecule in tissues with high glucose consumption (heart, brain, tumours), deposits may also be produced in other sites that are not of interest, such as points of inflammation. Nevertheless, FDG can be obtained commercially very easily. The use of PET is limited by a poor access to other types of radiolabelled drugs specific for other applications (synthesis of proteins and nucleic acids, hypoxia, immunoPET, etc.); even though such drugs have been developed or are being developed, access to them is difficult if one does not possess a cyclotron or does not have one nearby. Drugs radiolabelled with other isotopes (Ga-68, Cu-64, I-124) are also being developed. At the moment the main applications of PET, both in research and in the clinic, are oncology for localizing and monitoring tumours, and cardiology and neurology for the detection of specific pathologies[2].

Magnetic Resonance Imaging (MRI)

This imaging technic is based on the recording and interpretation of the electromagnetic signal emitted by the atomic nuclei, mainly the protons or hydrogen nuclei, in an external magnetic field.
The resulting image, increase the anatomic information of low RX contrast tissue, or soft tissues. By means of special equipment, physiological information can be obtained too.

Computerised Tomography (CT)

The images of computerised tomography are based on the different absorption by tissues when X-rays pass through them. CT is not a technique that provides information at the cellular or molecular level *per se*, and therefore it is not considered a molecular imaging technique. Nevertheless, this technique is of great importance for the anatomical location of functional images (PET and SPECT) and for their reconstruction. This is even more so if one takes into account that the molecular probes are increasingly specific. The radiation deposited in the experimental animals is not insignificant, which limits obtaining images in series[3].

2. DESING OF THE UNIT OF MOLECULAR IMAGING OF LARGE ANIMALS.

2.1. Radioactive Facility

The development of the imaging techniques mentioned above (PET, CT) implies the handling of non-sealed radioactive isotopes and the use of equipment emitting ionizing radiation (RX), which is why, according to the Spanish legislation[4,5], it is necessary to have a radioactive facility (RF) authorized by the competent authorities.
There is a central laboratory designed for the manipulation of high activities (limits of activity per essay from 10 to 30 mCi, depending on the type of isotope) or high-risk isotopes (volatile iodides, high energy gamma emitters). This laboratory is also used for the management and

control of the RF and contains the central radioactive waste depots. The Molecular Imaging Unit (MIU) is considered as another authorized area, to which the appropriate measures of control and protection are applied, based on the types of isotopes handled and the techniques developed[6].

PET Radioisotopes Central Laboratory.

During the design of the central PET lab, we take in account the activity, physical properties and how they are handled inside the lab.
The Lab is classificated as controlled with irradiation and contamination risk, the access to the lab is via a personnel airlock marked as vigilated with irradiation and contamination risk, with controlled doors with ID Cards keylock.
The cascaded negative pressure air control system guarantees the absence of contaminated traces in the other rooms of the lab, in case of incidents with volatile substances.
The lab furniture had working surface made of easy cleaning, and using single use absorbent materials.
At this lab we receipt, handle and dispense the radiopharmaceutical and radioisotopes provided by an authorized PET center. The radioactive waste materials is recorded, and aconditioned and stored until decay in a special room.
Inside the Pet Lab we have RP devices PET hotcell with laminar flow, activimeters , shielded bins, shielded containers for vials and syringes, radiation and contamination detectors, decontamination material for emergencies.

Molecular Imaging Unit for large animal.

The MIU must provide to the research groups with the latest imaging techniques. This unit must be in charge of the preparation of the doses, administration to the animals, obtaining the images, and their analysis. It must therefore have specifically trained personnel. Because of the nature of the work done, this unit must be intimately linked to the Animal Facility, ideally being located close to this facility. The unit must also have specific facilities both for handling the animals, which must form part of the Animal Facility, and for the analysis of the images, which should be outside the facility but close to it.

Specifically the UIM for big animals it is constituted by a lairage area for the animals of study, one lab for the dispensation of dose, the PET-RM imaging room, PET-CT imaging room, X-rays imaging room and operating room.

2.2. Design criteria for MIU, pigs section

The National Center of Investigation Cardiovascular (CNIC) is going to possess the systems of cardiovascular image multimodality more outposts, giving the exceptional opportunity to concentrate the pre-clinical and clinical reasearch by means of equipments of image multimodality. The animal model that is going to be used principally in the UIM is the pig. Due to the fact that the CNIC has vocation translational, It has been elected the porcine model by his anatomic-physiological similarity to the human being.

In addition, this animal model can be evaluated by the same technology that the human being (not invasive technologies of image, or invasive, and surgical procedures). But likewise, he presents major difficulty at the moment of designing the facilities and defining the protocols in Radiation protection issues. The investigative equipment of the center has wide experience in studies of image with models of big animal[7,8,9,10].

The animal will be kept in independent cages inside the animal zone before the procedure. The operator will enter the room and will realize an anaesthesia with intramuscular injection to be able to realize the manipulation of the animal under safety conditions. Later the pork will be transported to the room of preparation. Where one technician will proceed to the orotracheal

intubation and insertion of catheters in both marginal veins of the ear. Using one of them the anaesthesia will be kept.

A vesical sounding will be realized for continuous withdrawal of urine, in order that this way, once incorporated the radioisotope, the potentially contaminated urines will remain contained in a shielded container avoiding the risk of contaminated fluids. Once prepared, the pork will be transported in a stretcher adapted up to the zone of injection of isotopes next to imaging room. For the inoculation of the dose of the radioactive material, the operator an apron and he will have shielded protectors for syringes.

The doses will be almost the same that are in use in human studies (fitted to the weight of the animal) and one technician will proceed to the injection of the radiotracer across the marginal vein of the ear. After the period of incorporation of the marked molecule, He will pass to the animal to the imaging room to acquire the corresponding studies. Once finished the image acquisition, the pork still marked with the isotope, but after incorporation and imaging acquisition time, will be transported to cells different from the original ones. These cells will be located in an independent lairage room conveniently shielded.

The animal will get in one of the individual cells where mobile screens will be placed for his shielding. One technician will withdraw the intubation and venous catheters, as well as the urinary probe and in the cubicle the animal will awake (these cells in addition have an individualized system of management of effluent radioactive that will be controlled by Radioprotección's Service). Once passed the period of decay of the radioisotope, the animal will be transported to his conventional cage and there will be realized the radiological operational vigilance like that as the biological decontamination in the previous cell to make her prepared for the following occupation.

There exist protocols in which the animal sacrifices itself immediately after the radioactive material has decayed for pathological studies. Other animals will be used for experiments of long term by periodic studies of image. In this case, the animals will be submitted to periodic injections of isotopes for imaging studies. The maximum used dose will be always a minor that the one that could induce deterministic effects (physical hurts on tissues). In any case, all the experiments will be submitted to a committee of animal well-being that will value the doses of radiotracer that will be injected to the animal. In case some animal has to be sacrificed for any reason before the radioisotope has declined, it will be introduced in an individual cold chamber (well to 4ºC or-20ºC) and shielded, up to the radioisotope decay. Later the body of the animal will belong dedicated to the authorized manager.

2.3. Equipment

The area of animal manipulation will have to contain the necessary equipment for the development of these techniques. Below we will list the most important equipment to consider.

- Imaging equipment: PET/CT y PET/ NMR.
- Anaesthesia Table: sized according to the type of study animal.
- Shielded screen for the protection of the personnel during the injection and later period of incorporation of the radiopharmaceuticals.
- System for the manipulation of the sedated animal is important to consider PR's implications and the characteristics of the way, especially in RM (in high intensity magnetic fields).

Central PET lab area:

- Manipulation cell: easily decontaminatable stainless steel, with access for hands and material, shields adapted to the activities to manipulate (50 mm Pb. is usually sufficient), with an adequate system of air circulation and filtration if the type of isotope

to be manipulated requires it, and sufficient illumination. It is very recommendable to include a properly shielded housing for the ionization chamber of the activimeter.

- Dose dispensing system.
- Activimeter: appropriate for the isotopes to be used and in the range of the activities handled in the laboratory.
- Shielded carrying box for internal dose transport
- Tungsten syringe shielding.
- Trolley for internal dose transport.
- Tweezers of suitable length 20-35cm
- Radiation Monitor: with a probe sensitive to the levels of design of the facility, typically from 0.1uSv/h to 20 mSv/h
- Contamination Monitor: with a probe suitable for the emission of the isotopes of the facility and with a surface not less than 100cm2.
- Wastebaskets or furnishings for temporary waste storage: easily decontaminatable and with sufficient capacity for the amount of material to be used.

Lairage Area for injected animals:

- There area of individual systems for controlling the liquid wastes of the animals, which consists of a tank with a remote operated valve.
- Shielded screen for the protection of the personnel.
- Radiation Monitor: with a probe sensitive to the levels of design of the facility, typically from 0.1uSv/h to 20 mSv/h
- Contamination Monitor: with a probe suitable for the emission of the isotopes of the facility and with a surface not less than 100cm2.

2.4. Personnel. Functions and classification

The personnel implied in the manipulation of PET isotopes and radiation emitting equipment is as follows:

- Personnel in charge of Radiation Protection management: Made up of the Responsible of the RF (Supervisor in charge or Head of Radiation Protection), and a team of assigned technicians. These personnel will be in charge of the reception and registration of the commercial radioactive material and the operations of monitoring and control of the RF.
- Molecular Imaging Unit: Made up of a Person in charge holding a Supervisor license and a team of technicians with operator licenses, all in the field of application of the RF at issue. These personnel will be in charge of the development of the different imaging techniques that involve treating animals with radioactive material or handling radiation emitting equipment, including labelling the animals, the acquisition of images, and their subsequent analysis. This will be the only personnel authorized to carry out this type of techniques.
- Research Personnel: They will carry out the techniques of labelling experimental tracers with PET or SPECT isotopes. Depending on the activities and isotopes handled, they may or may not need to hold an operator license in the field of application of the RF at issue.

As a general norm, all exposed personnel will be considered as exposed workers of category B (according to the Spanish legislation), since it is improbable that they will receive doses above 6 mSv or 3/10 of specific limits in a calendar year. The technician who dispense and inject the radiotracers to the animals may be classified as

occupationally exposed personnel of Category A (who may receive doses above 6 mSv or 3/10 of specific limits in a calendar year).

2.5. Dosimetry

Dosimetry of occupationally exposed personnel of Category A and B must be done by means of individual dosimeters. In our case, we use thermoluminescence dosimeters. The whole body dose must be controlled during the entire working day by means of lapel dosimeters. For the personnel who directly handle the radioactive isotopes, the hand dose must also be controlled by means of ring dosimeters. After an accident an additional control by means of internal dosimetry must be done.

3. SETUP CRITERIA

3.1. Work norms

For working with PET isotopes and the radiation emitting equipment, the general norms for working with radioactive isotopes apply. These include norms regarding the operator: personal protection (gloves, lab coat, etc.), abiding by the norms of hygiene, use of appropriate shields, correct use of the dosimeter, etc.; norms regarding the work area: signposting, order and cleanliness, containment, monitoring, access control, etc.; and norms regarding the surroundings: contamination and radiation monitoring and correct waste management.

Specific norms for PET isotopes must also be applied, such as the use of specific movable shields (screens, dose dispenser, syringe protectors, etc.) or the use of means for increasing the distance between source and hands (forceps).

To guarantee the correct fulfilment of the norms and hence the protection of the operators, operational monitoring of the facility must be done by means of periodic inspections of control of the fulfilment of the operational norms, both in the central laboratory and in the authorized areas, including the MIU. These inspections include monitoring of contamination, waste management, appropriate conditions of order and cleanliness, filling in of the registers, correct maintenance of the specific monitors, etc. These inspections are carried out by the personnel in charge of the management of Radiation Protection.

3.2. Waste management

The radioactive waste of PET isotopes generated due to the accomplishment of planned works carried out, to incidents (accidental spillages, waste from marked animals, etc) and the cleanliness of material and areas of work.

The solid residues and liquids produced will be stored in the own containers of withdrawal (bottles of polypropylene in shielded container, shielded bin, etc) and stored in a location prearranged of the laboratory up to its decay (normally between 24 and 48 hours).

Also there exist zones of temporary storage for the morgue of animals where they will remain up to their decay and for radioisotope vials spent together to their commercial containers. The cutting material and the hypodermic needles used for the injection of radiotracers, they will settle in approved standard containers.

These containers will be shielded by another container up to his decay. In the lairage rooms destined to marked animals, there is a system adapted for the containment of urines and contaminated excrements. Effluent will remain confined up to his total decay; later to be evacuated to the network of sewer by means of a remote operator controlled electrical valve system.

The conditioning, record and evacuation of these residues will be realized by the responsible Supervisor or by the Operators of Radioprotection's Service. For it, for a correct management, the users of the UIM must identify all the units of containment generated with adhesive labels. In every label it will be indicated: radioisotope, physical condition, laboratory of origin, date of filling, name and surnames of the person who closed the packing, as well as if exist some chemical and / or biological additional risk.

3.3. Biosafety

Besides the risks and procedures that the work carries with animals marked with radioisotopes in the MIU, it is necessary to contemplate the tests that combine with the inoculation of microorganisms of the Risk Group 2, or it is possible the situation where an animal is infected by some microorganism and this of natural form could suppose some risk of transmission to the scientific staff and to the personnel in charge of the animal care area. For this reason it is fundamental that exist Biosafety´s procedures that are destined to reduce these risks. The rooms and stays where biological risk exists more are those in which after the studies of image, the animals remain in a space of lairage, shielded and separated.

The excrements and the urines are gathered by means of a suitable system of containment where besides the decay of the radioisotopes, a treatment of inactivation is realized by means of wide spectrum germicides. The rooms of autopsies and operating rooms are another critical point at the moment of containing the transmission of infectious micro-organisms so that these rooms are designed so that they are easy to clean and decontaminate.

In case of big animals marked with radioisotopes and inoculated with micro-organisms of from risk group 2, who die during a study or are sacrificed, follows a specific procedure of waste management and decontamination. The autopsies are realized as soon as the radioisotope has decayed and till then the body of the animal remains in a cold chamber, shielded with mobile screens. The remains of corpse of the animal and his effluent ones are gathered in approved, closed containers. The generated containers are processed by autoclave in the own installation and later they are withdrawn by a managing authorized company, which will incinerate.

The bed materials and the materials of decontamination generated, they will retire so that there diminishes the production of aerosols and powder. All the surfaces and materials are cleaned and decontaminated applied by atomizers. These works need the utilization of identical Equipments of Personal Protection to the existing risk (workwear, masks, footsore, double glove, etc.) that must move back and autoclaved once finished the work. There will be given specific formation of the whole implied personnel it is these tests.

3.4. Training

Besides knowledge acquired in training courses for operators or supervisors of Radioactive Facilities, all personnel involved in handling PET or SPECT isotopes must receive specific in-house training. This training will consist of giving different seminars in which information will be included on the general safety norms for working in a laboratory, the handling of radioactive PET/SPECT isotopes (radiological hazard, use of shields, detectors, etc.), operation, and the Emergency Plan of the RF. The personnel of the Imaging Unit dedicated to labelling the animals will receive additional training in handling research animals. These personnel must also first do practical and specific training, developing the techniques of animal handling under the same conditions as when using radioactive material, for example using portable shields (screens, syringe protectors).

4. CONCLUSIONS

The research in cardiovascular image in big animals joins in an alone imaging device two promising technologies of image that even had not been achieved to join still. It is a question of the merger of the PET and of the RM. The RM, it has demonstrated his capacity not only of being precise in the anatomical definition but also of defining the structure of the tissue of form superior to the one that achieves the CT for low X-Ray contrast tissue. In addition, the RM does not issue radiations since there do the devices that use X-rays. It is logical, therefore, that the combination of both methods finishes not only giving good results but it is very profitable to level of the Radiological Protection. Nevertheless, to make possible the merger PET/RM it has been necessary to settle some technical disadvantages that were disabling a combination of these characteristics.

Between them it is outlined to achieve that many of the components of the PET work in the environment of a high magnetic field as the one that is generated by the RM. On the other hand to improve the quality loss of the images that obtains the resonance when in the same machine it is necessary to connect a PET. And finally the creation of a software that allows the simultaneous compatibility of both technologies.

The personnel dosimetry approach, for the ones who handle the animals, the vials or syringes with the doses of radiotracer the aim is to keep the received dose as low as possible. In the work with big animals one tries to obtain levels of doses lower than the personnel that realizes these technologies in the clinical area, since the doses used for big animals are in the order of the doses applied to human beings.

The previous cold training, as well as the selection of personnel are vital actions to reach the target of the safe use of these isotopes and to support the low doses. One has seen that is preferable to give priority to the previous experience and formation in the managing animal, which will be complemented by the formation in PR. The work with big animals complicates very much the design; the system must be studied thoroughly all affected to establish the most suitable means (shielding, stock of animals, control of effluent, etc.) to every case.

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