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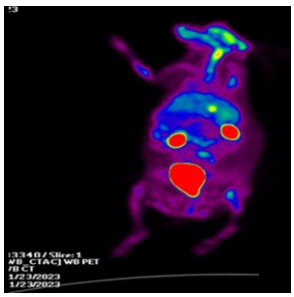
नए रेडियो-भेषजिकों के पूर्व-नैदानिक विकास में प्रमुख तकनीकी बाधाएं : एक परिप्रेक्ष्य

योगिता शेटे एवं संदीप बसु

विकिरण चिकित्सा केंद्र, भाभा परमाणु अनुसंधान केंद्र, टाटा स्मारक केंद्र एनेक्स बिल्डिंग, परेल, मुंबई-400012, भारत



खरगोश में जैव वितरण प्रतिबिंब विशिष्ट क्षेत्रों में उच्च तीव्रता वाले उद्ग्रहण का चित्रण।

सारांश

नए रेडियो-भेषजिक विकास एवं विस्तार में, नैदानिक संरक्षा मानव अनुप्रयोग के लिए मौलिक है। नैदानिक या चिकित्सीय उपयोग के लिए रेडियो-भेषजिक के पूर्व-नैदानिक विकास के चरणों में जीवशाला में जानवरों का उपयोग करके जोखिम मूल्यांकन एक महत्वपूर्ण कदम है। पशु अध्ययन रेडियो-भेषजिक विकास की आधारशिला है, जो 'कट एंड काउंट' डाटा एकत्र करने के लिए एक पारंपरिक विधि के रूप में कार्य करता है। वैज्ञानिक समुदायों ने दुनिया भर में पशु कल्याण और पशु प्रयोगों के लिए 3 (R) आर सिद्धांत को अपनाया है। इसलिए, पशु मॉडलों की वाइवो/जीवस्थ जैव वितरण और भेषज-गुणगतिकी समझ में दिनचर्या का उपयोग करके नए रेडियो-भेषजिक के परीक्षण को अपनाया गया है। नए रेडियो-भेषजिक विकसित करते समय पशु शरीर विज्ञान और प्रयोगात्मक अभिकल्पन का मौलिक ज्ञान आवश्यक है, क्योंकि यह ज्ञान प्रजातियों, उपभेदों, लिंग और उपयोग किए गए जानवरों की कुल संख्या के चयन का औचित्य प्रदान करता है। इस रिपोर्ट में, हमने सामाजिक लाभ के लिए वैज्ञानिक परिणाम सुनिश्चित करने हेतु नैतिक दिशानिर्देशों का पालन करते हुए, नई रेडियो-भेषजिक के विकास में बाधाओं को दूर करने के लिए अपनाई जाने वाली व्यावहारिक चुनौतियों और उनके समाधानों का उल्लेख करने का प्रयास किया।

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Key Technical Barriers in Pre-clinical Development of New Radiopharmaceuticals: A Perspective

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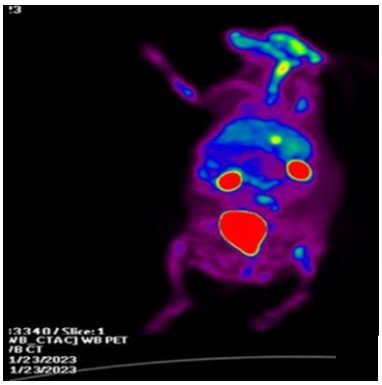

Biodistribution imaging in rabbit displaying high-intensity uptake in specific regions.

ABSTRACT

In the new radiopharmaceutical development and expansion, the clinical safety is fundamental for human application. Risk assessment by using laboratory animals is a crucial step in the phases of pre-clinical development of radiopharmaceuticals either for diagnostic or therapeutic use. Animal studies are a cornerstone of radiopharmaceutical development, where 'cut and count' acts as a traditional method for gathering data. The scientific communities have embraced the animal welfare and 3Rs principle for animal experiments worldwide. Therefore, testing of new radiopharmaceuticals by using routine in vivo biodistribution & pharmacokinetic understanding of animal models have been adopted. Fundamental knowledge of animal physiology and experimental design is essential when developing new radiopharmaceuticals, as this knowledge justifies the selection of the species, strains, sex, and total number of animals used. In this report, we endeavoured to address the practical challenges and their solutions to be implemented to overcome the barriers in the development of new radiopharmaceuticals, while adhering to ethical guidelines to ensure scientific outcomes for societal benefit.

KEYWORDS: *Nuclear medicine imaging, Laboratory animals , Radiopharmaceuticals*

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Introduction

Biomedical and pharmaceutical research relies extensively upon the use of laboratory mice, wistar rats, Guinea pigs, and rabbits (Shete et al., 2024). Administering investigational agents such as novel drug formulations, antibodies, and radiopharmaceuticals allows researchers to determine vital pharmacokinetic and toxicological data necessary for successful clinical translation (Turner et al, 2011).

The Radiation Medicine Centre, BARC, Parel, is a pioneer institute in radioisotopic treatment in India. Although 1963 marked the beginning of preclinical radiopharmaceutical testing in animals, specific guidelines for handling radioactivity in this context were initially underdeveloped. Subsequently, the Atomic Energy Regulatory Board was established to regulate the safety and use of these materials. The AERB (Atomic Energy Regulatory Board) and International Atomic Energy Agency (IAEA), have established guidelines for optimizing medical radiation exposure and setting safety standards for human patients. The evaluation of preclinical animal studies in radiopharmaceutical development hinges on three main factors, translational relevance to human application, the physicochemical properties of the agent, and the specific study objectives (Korde et al., 2022). The persistent advancement of radioactive tracers is imperative to address the complex demands of modern health challenges. As a result, non-clinical testing of radiopharmaceuticals has experienced a threefold increase, leading to a surge in animal usage.

Radiopharmaceutical studies aim to ensure safe clinical use through the evaluation of in-vivo behaviour. Essential nonclinical studies must be performed to define target engagement, non-target organ uptake, and biodistribution, ensuring safe administration (Baldrick, 2021., Korde et al. 2022). Various factors, including species-specific variations, difference in metabolic pathways or protein expressions, administration routes, distress, method of evaluation and anesthesia, influence experimental outcome, often resulting in failed clinical trials. A literature survey highlights that improper consideration of these factors contributes to significant delays in translating preclinical findings to human applications. (Andes et al., 2002, Namdari et al.,2021, Mukherjee et al., 2022, Mizuo et al., 2023).

Globally new guidelines have been established, stating that the use of animals in experiments must adhere to the 3Rs principle of Replacement, Reduction, and Refinement (CCSEA,2018, Kousholt et al., 2023). Furthermore, the IAEA preclinical testing guidelines (2023) emphasize that the '3Rs' principle must be followed in accordance with national guidelines. In biodistribution studies, the principles of reduction and refinement can be achieved by utilizing advanced imaging techniques and modifying evaluation methods.

Despite significant scientific progress, regulatory frameworks for preclinical radiopharmaceutical animal testing have failed to evolve, adhering instead to protocols established several decades ago. Consequently, a comprehensive revision of the Radiopharmaceutical Committee (RPC) regulations governing preclinical studies for radiopharmaceuticals is required.

We herein outline the essential technical considerations for in vivo radiopharmaceutical studies, covering the various component such as anesthesia, dosing, animal model selection, and appropriate evaluation method to obtain biodistribution data.

To achieve better scientific outcomes from the animal experiments, it is necessary to integrate fundamental knowledge of existing animal species and refine procedures, which leads to improvements in both scientific research and animal welfare.

Technical Hurdles

Routes of administrations

An objective of the animal experiments, the route of administration is defined in preclinical models by: (i) the properties of the compound, (ii) nature of the formulation and (iii) routes of human application. Different routes of administrations like intravenous, intramuscular, Subcutaneous, intraperitoneal and intradermal, intrathoracic, intranasal, intracerebral and topical used in animals. The most commonly employed routes of administration for preclinical test substances are intravenous, subcutaneous, intraperitoneal (Shimizu, 2004). Administration methods for radiopharmaceuticals are tailored to their physicochemical properties and experimental findings. Precise intravenous administration is crucial for the success of many preclinical radiopharmaceutical studies (Vines, et al., 2011). However, radiopharmaceutical was also tested by subcutaneous, retro-orbital, intraperitoneal administrations are also examined by investigators in preclinical studies of radiopharmaceuticals (Schiffer, et al., 2007, Fueger et al., 2006).

The administration route in animal research is usually kept consistent with that used in humans, while the specific dose of the test radiopharmaceutical is adjusted according to the intended application. While in majority the preferred route of administration for radiopharmaceutical is intravenous route, there are few exceptions like (a) Iodine-131 is given by oral route, and the same for the Technetium-99m for gastric emptying study, while (b) inhalation route is adopted for radiolabeled gases used in lung studies include xenon-133, Krypton-81m (Clerc et al., 2017).

Administering radiopharmaceuticals via the fragile lateral tail veins of conscious mice is a technically demanding procedure due to the small, delicate nature of the vessels (Nanni et.al.,2007). The small diameter of the lateral tail vein often leads to vessel damage and blockage when repeated injections are attempted (Shimizu.,2004). These difficulties not only complicate subsequent intravenous access but also cause pain and distress, impacting the animal's welfare and altering normal physiological parameters. Consequently, this can affect group behaviour and introduce variability in the biodistribution data, compromising study integrity (Vines, 2011, NIH guideline, 2019).

Handling stress is a major factor influencing vein accessibility in rodent models. Untrained personnel, excessive

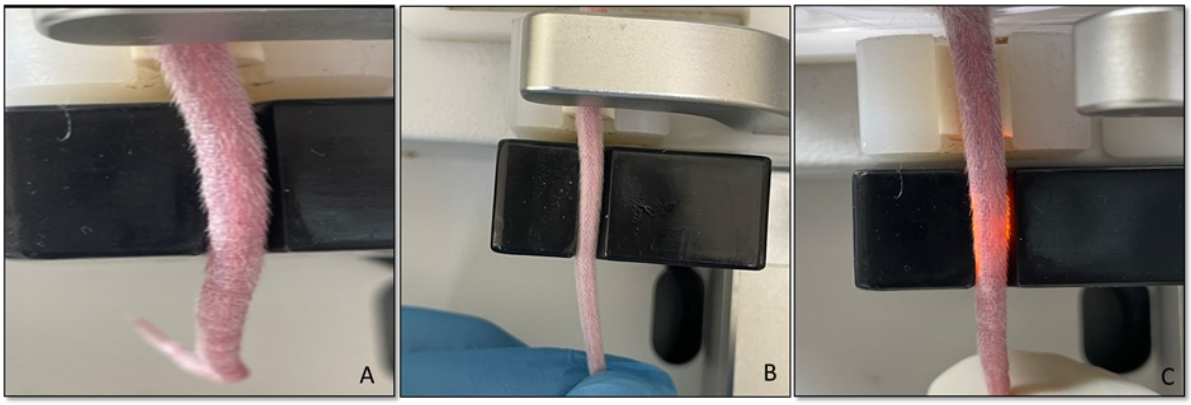

Fig.1: Intravenous administration of radiopharmaceuticals via mouse tail vein in preclinical testing: A. Tail vein in normal condition, B. After 30 minutes in a 37°C warming chamber, the tail vein showed improved visibility compared to normal conditions, C. The combined use of warming and a tail vein light illuminator improves vascular visibility in BALB/c mice.

handling, and environmental noise can trigger stress in mice and rats. This leads to increased aggression, fear-based freezing, and significant peripheral vasoconstriction, creating substantial challenges for successful, intravenous injections (Kim et al., 2019, Kiyatkin et al., 2021).

Radiotracers are frequently administered into the interstitium rather than accurate intravenously, negatively affecting preclinical research outcomes. The frequency and severity of these mistakes, even with experienced staff, are often underestimated, serving as a critical limiting factor for data quality in small rodent PET-CT imaging studies (Groman et al., 2004). Radiopharmaceutical biodistribution and PET quantification in mice are significantly influenced by injection technique, leading to discrepancies in percent uptake values in mice (Vines et al., 2011).

Apart from this, the radioactivity handler person gets the unnecessary exposure when manipulating the tail vein for radiopharmaceutical injection in the animals (Vines, 2011).

To overcome such problems, animal handlers should receive comprehensive training in effective handling techniques to minimize stress and distress in animals. A proper way of handling animals reduces aggression and enhances their normal physiological state, which in turn helps ensure the accuracy of intravenous injections.

To prepare the tail for improved visibility and promote vasodilation, consideration can be made for using a heating lamp, warming chamber, immersing it in warm water, or utilizing a specialized platform with a heating element (Shimizu., 2004) (Fig.1, 2, 3 and 4).

While the tail vein is the conventional route for intravenous injections in small rodents, scientific literature recommends several alternative, highly accessible blood vessels to improve injection accuracy and success rates. These alternatives include the dorsal metatarsal vein of the hind limb, the saphenous vein, the external jugular vein, and the retro-orbital plexus (Wang et al., 2022).

To enhance the accuracy of nonclinical data in radiopharmaceutical studies and better mimic common human administration routes, appropriate intravenous injection techniques in small animals must be considered, even if these routes are not routinely employed in standard laboratory practices.

Selection of animal species

The selection of an appropriate animal model is a key factor defining research outcomes, with the optimal choice depending heavily on the study's specific objectives. Proper species selection based on the therapeutic or diagnostic

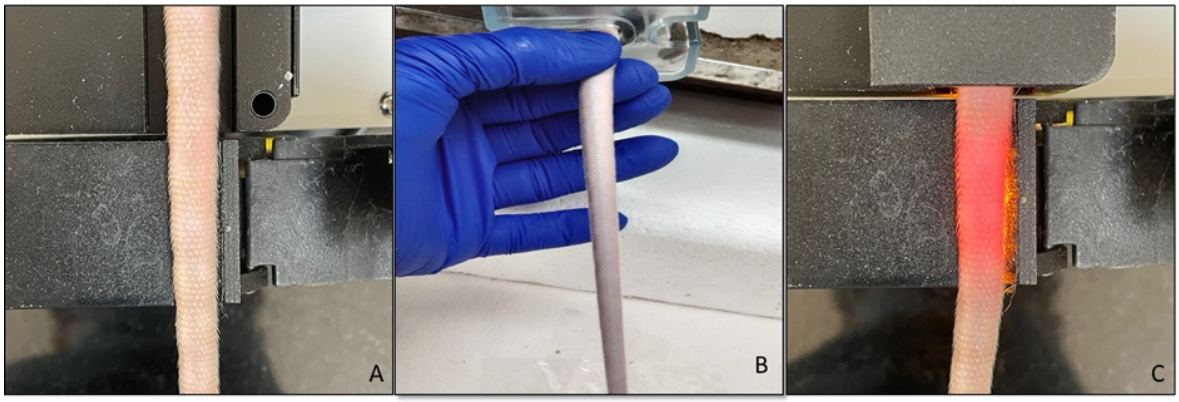

Fig.2: Intravenous administration of radiopharmaceuticals via Rat tail vein in preclinical testing: A. Rat tail vein in normal condition, B. After 30 minutes in a 37 °C warming chamber, the tail vein showed improved visibility compared to normal conditions, C. The combined use of warming and a tail vein light illuminator improves vascular visibility in wistar rat.

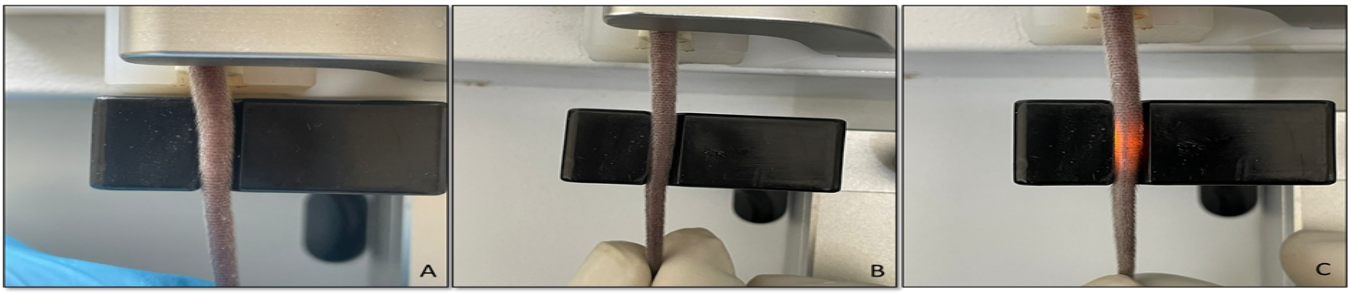

Fig.3: Intravenous administration of radiopharmaceuticals via C57Bl/6 mice tail vein in preclinical testing: A. Black mice tail vein in normal condition, B. After 30 minutes in a 37°C warming chamber, the tail vein showed improved visibility compared to normal conditions, C. The combined use of warming and a tail vein light illuminator improves vascular visibility in black mice.

application of radiopharmaceuticals is essential for enhancing the translation of animal data to humans (Alstrup et al., 2021, Fowler et al., 2001).

Animal species in nonclinical studies are chosen for their similarity to humans in anatomy, physiology, and metabolism specifically, "Absorption, Distribution, Metabolism, and Excretion" (ADME). This selection ensures that cross-reactivity and pharmacological responses are relevant for human clinical application (Namdari et al., 2022, Mizuo et al., 2023).

Generally, mice and rats are the most commonly used animal models for radiopharmaceutical studies, often requiring a large number of animals to be sacrificed to generate biodistribution data for each time point. Beyond these two, other species are also being explored for testing the same radiopharmaceuticals and for various other studies (Alstrup et al.,2021, Lambrecht., 2012, Kuntner et al., 2011, Maitz et al., 2024), (Fig.5).

The primary objective in radiopharmaceutical studies is to ensure human safety by providing safe administration, preventing systemic reactions, identifying target and non-target organs, and determining retention and excretion times. There are no clear, universal guidelines for species selection in nonclinical safety assessments. The Japanese guidelines recommend that non-clinical regulatory toxicology studies be conducted in two species (one rodent and one non-rodent) with proper justification. (Stonerook, 2015., Namdari et al., 2022). However, the IAEA guideline (2023) suggested

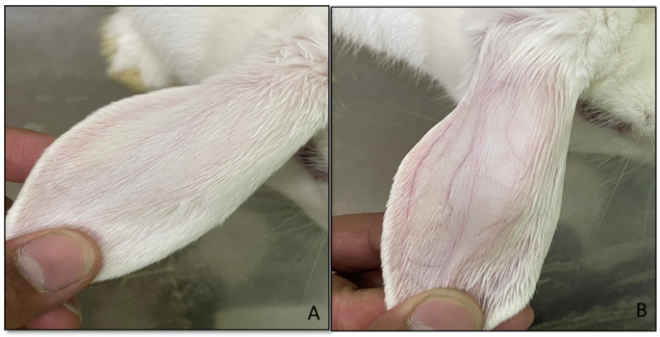

Fig.4: Intravenous administration of radiopharmaceuticals via New Zealand White Rabbit Ear vein in preclinical testing: A. Rabbit marginal and central ear vein easily seen in normal condition, B. Simple tap and alcohol rub for few minutes increase more clear visible ear vein in normal condition in Rabbit.

radiopharmaceutical evaluation in both sexes in a single animal species is sufficient, unless the planned indication is gender-specific.

General toxicology typically utilizes small rodents and non-rodent species, however, these models are often restricted in dynamic scanning applications due to limited blood volume, rapid induction of hypothermia, smaller body size and poor tolerance to anesthesia (Krinke., 2000). Comparing multiple tracers in a single animal is difficult, necessitating the use of more rodent animals across different groups. Unfortunately, this conflicts with the 3Rs, the reduction replacement and refinement principle. Additionally, the limited spatial resolution of PET and SPECT scanners, relative to the small anatomy of mice and rats, challenges the recognition of detailed anatomical structures (Stadelmann et al., 2015). As per the IAEA, guideline 2021, Hematology, serum biochemistry test should also include along with biodistribution and dosimetry studies. Due to the limited blood volume in mice and rats, sufficient samples cannot be frequently obtained for the required tests to meet the guideline.

A non-rodent or larger animal models facilitate in-depth experimentation and the utilization of human clinical PET scanners. Due to their substantial body size and blood volume, these models are ideal for dynamic studies, with prolonged anesthesia facilitating longitudinal comparisons of multiple tracers in a single subject (Alstrup et al., 2009). As an intermediate-sized model, the rabbit offers a superior platform for radiopharmaceutical evaluation, providing clear visualization of major organ systems including the brain, heart, lungs, liver, kidneys, thyroid, and lymph nodes along with detailed skeletal mapping. Rabbits are a suitable translational model for nuclear imaging, as PET and SPECT data acquired with human-equivalent protocols show strong similarity to human results (Duranthon et al., 2012, Gebhardt et al., 2025, Shete et al., 2025).

Considering the current scenario, small rodents have multiple limitations and translating radiopharmaceutical data from animals to humans requires careful consideration and data obtained from rats and mice should be cautiously interpreted (IAEC, 2023).

To advance radiopharmaceutical evaluation, we must incorporate non-rodent models like rabbits, ferrets, and dogs. Rabbits are particularly advantageous, offering a superior balance of technical feasibility, manageable laboratory

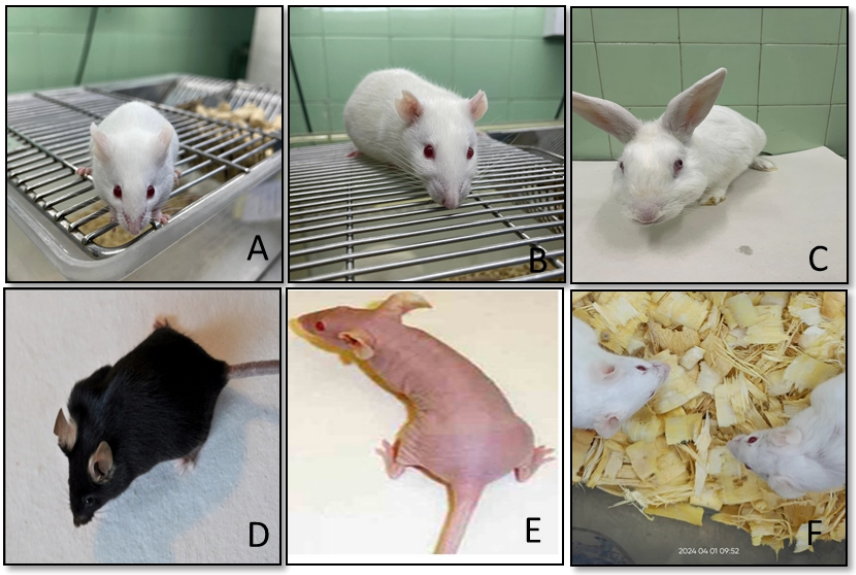

Fig.5: Different Animal Species Utilized for Radiopharmaceutical Development in non-clinical testing in Radiation Medicine centre Animal House Facility, Parel. A. BALB/c mice, B. Wistar Rats, C. NewZealand White Rabbit, D. C57Bl/6 mice, E. Nude mice, F. Severe Combined Immunodeficiency(SCID) mice.

housing, and straightforward experimental handling of radioactive agents. These large animal models bridge the gap between preclinical studies and human application by improving model accuracy. Compared to smaller rodents, rabbits provide superior experimental options, such as convenient auricular vein access for serial administration and blood sampling with reduced stress. Performance of different haematology and biochemistry tests per the international radiopharmaceutical guidelines, 2023. Their size and physiology also make them ideal for long-term, anesthetized imaging and simplified physical dosimetry studies. Additionally, the use of rabbits enables strict adherence to the ALARA principle for radioactivity handlers during radiopharmaceutical injection, imaging, dosimetry as well as meeting the 3Rs principle as per the (CCSEA, guideline,2018, Shete et al., 2025).

Evaluation of system

To ensure ethical compliance and high-quality data, nonclinical testing of radiopharmaceuticals is shifting from traditional, terminal tissue analysis to advanced, non-invasive imaging modalities like micro-PET-CT and micro-SPECT-CT. These technologies allow for longitudinal in-vivo tracking within the same animal, reducing the total animal count while enhancing data accuracy. Adapting these methodologies is essential to refine the current procedures, minimize error and align with the highest standards of animal welfare (Klein et al., 2007) (Fig.6).

Dose

The required dose of radiopharmaceuticals varies based on several factors, including the study application, formulation, radioisotope half-life, and experimental duration. While human diagnostic dosages are strictly defined, there are no universally established guidelines for preclinical animal studies in India. Generally, intravenous injections for PET-CT or SPECT-CT imaging in rats and mice range between 100-500 µCi (microcuries), though some studies report successful imaging with doses as low as 50 µCi, depending on the isotope, system sensitivity, and desired image quality. For rabbits, recommended activity ranges from 3–5 mCi for SPECT-CT and 1–2 mCi for PET-CT (Shete et al., 2024). When imaging rabbits, higher activity concentrations in smaller organs are preferred to improve visibility across extended time points for particular study.

Ethical Consideration and Dosimetry

Accurately predicting human radiation toxicity from small animal data is still difficult due to significant differences, such as faster metabolism and higher dose requirements in rodents. The situation is further complicated by the need for large animal numbers to accurately trace RP clearance. Given this scenario, an ideal experimental model must provide, in a single study, complete data on biodistribution, excretion, and detailed target and non-target organ kinetics. To improve ethical standards in preclinical studies, focusing on a few large animal models rather than hundreds of small rodents is preferred for studying biodistribution (Kiani et al.,2022, Shete et al., 2025).

Regulatory Approvals

Pre-clinical studies involving radioactivity in animals necessitate two key approvals in India, IAEC approval for animal experimentation (under CPCSEA) and, for radioactive material usage, licensure of laboratory premises by the AERB. Researchers must adhere to the ethical guidelines prescribed in the IAEC proposal, though it is important to note that no fixed dose limits for radioactive administration in animals are prescribed in any of the committees. Before granting approval, the IAEC must rigorously review the scientific justification, the criticality of using animal models, and the appropriateness of the chosen species.

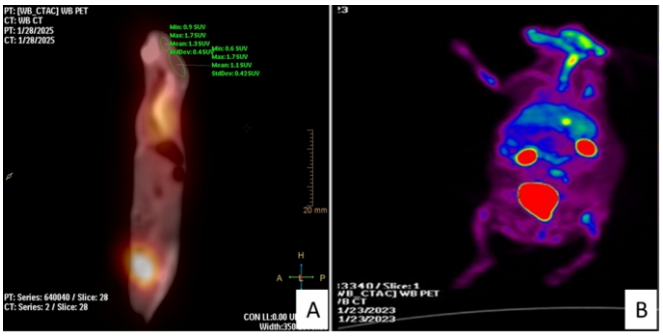

Fig.6: PET-CT Imaging in Mice and Rabbit with Fludeoxyglucose F18(18F-FDG) radiopharmaceutical comparative analysis: A. Although mice whole-body imaging showed specific uptake in the hot region, compartmental analysis was challenging to distinguish, B: Biodistribution imaging in the rabbit displayed high-intensity uptake in specific regions, enabling compartmental kinetic modeling across various tissues.

Radioactive waste handling and contamination control

Preclinical studies often generate significant amounts of solid and liquid radioactive waste. Following standard safety procedures, the 'delay and decay' method is an effective solution for managing contaminated surgical instruments, cotton plugs, absorbent sheets, and dissection materials. Generation of liquid waste should be strictly minimized or avoided. The second most important approach to radioactive waste management is confinement within designated areas. This will also ensure the restricted access and safe handling. Minimizing waste volume improves safety and operational efficiency. To ensure radioactive biomedical safety, the local Radiation Safety Officer must monitor all waste and disposal is authorized only upon approval, following standard procedures.

Conclusion

Preclinical animal data is a mandatory requirement by radiopharmaceutical regulatory authorities for approval of new radiopharmaceuticals, therefore, animal experiments have evolved as an essential part of the nonclinical study phase, However, adherence and commitment to the 3Rs principles (Replacement, Reduction, and Refinement) must be prioritized before its approval by the scientific community. Periodic revision of preclinical radiopharmaceutical guidelines is essential to adapt to new scientific developments. Preclinical studies must balance the pursuit of scientific goals with a rigorous commitment to animal welfare.

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