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Role of Advance Nuclear Medicine Imaging Techniques in Preclinical Drug Development and Three R's in Animal Research

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Abstract

Preclinical models have played a fundamental role in understanding the pharmacokinetics and biodistribution of novel drugs and radiopharmaceuticals prior to its clinical testing. Application of radiotracers and Nuclear Imaging Techniques have the potential of real time tracking of radiotracer molecules in a non-invasive manner. The three R's principles are now a worldwide commitment for laboratory animals. Hence, we have carried out PET/CT and scintigraphy imaging technique for monitoring and exploring the *in vivo* pharmacokinetic profiles of novel drugs in the lung, tumor site, stomach as well as their systemic retention in animals. The present work includes different case studies viz. Evaluation and gastric retention of radiolabeled metformin floating tablet in rabbits by gamma scintigraphy; and Normal biodistribution of radiolabeled etoposide-nano-formulation in New Zealand White rabbit and evaluation of anticancer activity in C57BL/6 mice as a lung metastatic melanoma tumor model. Results showed that the gastric retention of radiolabeled metformin floating tablet was observed to be 50% in the rabbit stomach up to 6h post administration and radiolabeled etoposide-nano-formulation showed 97% of lung uptake in normal rabbit at 1h and significant regression in the number of lung metastasis sites in mice tumor model. Successful drug delivery information can be obtained by *in vivo* monitoring which enables acquiring statistically significant data from a limited number of animals by the advance imaging modalities. These techniques have an added advantage to provide the 'Reduction' principle in laboratory animals and also boost the research and development for new drugs and radiopharmaceuticals.

Keywords: Laboratory animals, Drug delivery, Radiotracer, 3 R's, Nuclear Medicine Imaging

Introduction

Animal studies play an important role in basic and clinical research for application in patient management. As a result, both humans and animals are expecting longer and healthier life as compared to the earlier era (Siegel, 1994). Laboratory animals are playing a key role in physiological research and drug development in medicine and two third of studies carried out by noble laureates, have relied on animal data since 1901 (Rai and Kaushik, 2018). During drug development, toxicity, pharmacokinetic and biodistribution data is generated using animal model. Drug approving authority recommends the mandatory experimentation of the aforementioned studies in order to establish the safety margin (Morgan *et al.,* 2013). Large number of animals are dissected and sacrificed in order to obtain reliable preclinical data. Apart from this, wide usage of animals for development of models, carring out surgical procedures and testing of dermatological and cosmetic preparations has raised concerns about the ways animals are put to unnecessary pain, discomfort and the ethics involved in animal experimentation (Badyal & Desai, 2014). The study hypothesis propsed in research is confirmed to using different techniques and applications in preclinical models. Nuclear medicine imaging technique is one of the best *in vivo,* highly sensitive and quantiative method utilized on routine basis for diagnosis of different organ function in humans. The most commonly used radiotracers are Fluorine-18, Technetium-99m, Gallium-68 etc. These radiotracers can be used to label the drug formulation or a test compound administred *in vivo* for understanding the biodistribution pattern and pharmakonetics. The *in vivo* sequential static or dynamic imaging enables the real time tracking of radiolabeled compound in biological system (Wade *et al.,* 2013; Chaudhari, 2017). Imaging techniques are also useful for obtaining different time point data from same animal in biodistribution study, This significantly decreases the number of animals used in studies. Worldwide experiments in animals also encourage 3Rs priniciple in drug development (Magda and Goldman, 2012). Hence, more emphasis is given on reduction and replacement of the animal model (Wang *et al.,* 2017). Use of imaging technique provides benefit to the laboratory animal science for achieveing the 3Rs principle to a significant extent. Therefore, an attempt was made to improve

the judicious utilization of the animals with the help of nuclear imaging technique. Towards new drug development and understanding its delivery to-target sites, imaging techniques provide distinct advantages over that of invasive procedures involved in animal experimentation. Animal studies with *in vivo* imaging showed better outcome and interpretations of the data. Nuclear medicine imaging has diverse applications in the field of medical science with special emphasis in drug development and it helps to accomplish the reduction principle for laboratory animals.

Material and Methods

Animal Experiment: The animals used in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of BARC. Three IAEC approved projects (BAEC/01/14, BAEC/18/12) were included in the study. Oral drug delivery and lung delivery via intranasal route of newly developed drug formulation pharmacokinetics were carried out and *in vivo* tracking studies in animal models are also reported in this study.

1. **Oral drug delivery to stomach:** Healthy male New Zealand White Rabbits weighing 3.0- 3.5kg were used. Animal restraining was done by injecting Ketamine (22mg/kg body weight) and Xylazine (5mg/kg body weight) by intramuscular route. In all the delivery study models, the animals were placed in prone position. The gastric drug delivery metformin tablet (5mm diameter) was filled with a radioactive solution of ^{99m}Tc–DTPA (approximately 400- 600µci) and was sealed with molten gelucier. This radiolabeled tablet was used for oral administration and imaging was done from 0 min to 6 h post administration (Karpe *et al.,* 2015)

2. **Intranasal drug delivery for lung targeting**:

2.1. Imaging: ^{99m}Tc-labeled Etoposide anticancer drug formulation was given by intranasal route to rabbit with the help of nasal spray pump and imaging was carried out from 30 min to 6 h post administration. The sequential static scintigraphy imaging were carried out in all the cases by using a dual head gamma camera. Analysis of different studies were done by using the computer-based RAMLA reconstruction algorithm software system (Ghadiri *et al.,* 2019).

2.2. Biodistribution: C57BL/6 mice were used for development of lung metastatic tumor model by using $2x10^4$ B16F10 cells in 100µl, which were injected by intravenous route. Drug treatment to study its efficacy was initiated after 21 days with radiolabeled Etoposide nanoformaulation, once a week by intravenous route (Lohade *et al.,* 2015). Cell cytology and histopathology was performed in lungs tissue by standard method (Pawar *et al.*, 2017).

Result

A) Scintigraphy imaging study in New Zealand White rabbit:

1. **Oral route:** The gastric retentive tablet formulation was given by oral route followed by saline administrations in New Zealand White rabbit. As it was radiolabelled, it was possible to identify the location and clearance patteren of the formulation from 0 h to 6 h in the gastric region. 50% retention of the radiolabeled formulation was observed in the stomach at 6 h (Fig.1).

2. **Intranasal drug delivery of lung targeting chemotherapy:** ^{99m}Tc-labeled etoposide-nano-formulation showed notable regression in the number of lung metastasis sites (n=3) in the treated group of mice as compared to those (n=100 to 120) in control mice. The normal distribution and uptake was 97% at 1h in normal lungs of the rabbit (Fig.2). The cell cytology of metastatic lung tissue showed alveoli epithelial tissue with metastatic melanocytes having melanine pigment. Histology of the same tissue showed a clump of metastatic melanoma cells within and adjacent to lung alveoli and bronchioles.

Discussion

Animal models play crucial roles in the field of preclinical drug development and biomedical research (Youn & Hong, 2012). In the present study, we have compiled different types of drug delivery system and studied the efficacy of nuclear medicine imaging technique for the evaluation of drug delivery systems for targeting specific sites using minimum number of animals.

The radiotracer along with specific imaging techniques play an important role in *in vivo* tracking of the novel drug formulations targeted to specific delivery sites. It also helps in understaning of biodistribution in rabbits as well as the decreased pattern from the lung and stomach by intranasal and oral route, respectively. It was possible to do quantatitve estimation of radiotracer with respect to time in each animal study. This helped to understand the distribution pattern and successful delivery to target site. The real time tracking of administered formulation till its maximum uptake was possible due to imaging. This also reduced the number of animals required for calculating uptake at various time points.

An instant follow up of gastric retentive metforming floating tablet and its quantification was possible with continuous sequential imaging. The gastric retention of the formulation occurred due to swelling of polymers and the release of CO_2. This could easily be monitored by gamma scintigraphy imaging procedure at different time points in minimal number of animals (Karpe *et al.,* 2015). Animals used in this study were recovered sucessfully and the formulation was also excreted out after a washout period of 24 h. Hence, it is possible to reuse the same animal after obtaining due approval of the Ethics committee.

Animal biodistribution studies are recommended to establish safety data (Lima and Videira, 2018). Histological studies with modified nano Etoposide chemotherapy agent, tested in murine lung melanoma model, confirmed its *in vivo* efficacy. This findings were corroborated with lung uptake scintigraphy imaging. Specific drug deposition occurred due to their optimum partical size which facilitated the drug deposition at target site. Hence, nuclear imaging is a valuable and reliable technique and significant data can be obtained with minimal number of animals.

Conclusion

A proper study design towards identification of a successful drug delivery system is possible with nuclear imaging techniques with minimal usage of animals. It also gives additional insights about physiology, pathology, biodistribution, metabolic status of tissue and behavioral patterns of novel drugs for future clinical applications.

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Figures:

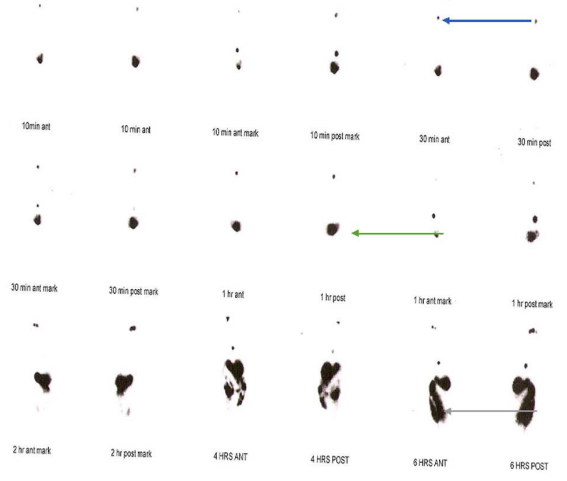

Figure.1. Peroral Gastric retention in Scintigraphy Image: Gastric retention of radiolabeled formulation (Green arrow), oral part of the rabbit with (blue arrow) drug formulation image from 10 min to 6hrs, 50% retention observed at 3hrs and slow release in the intestine at 6hr (Grey arrow).

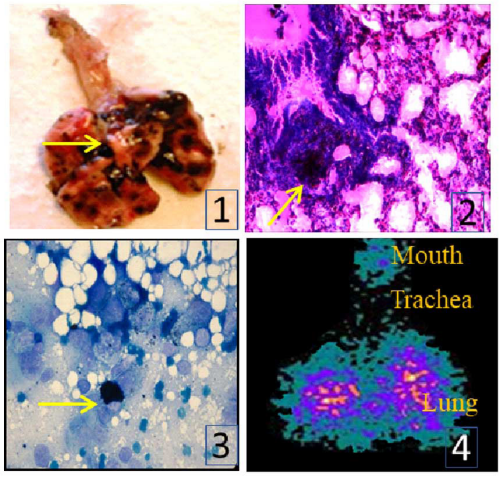

Figure.2. Intranasal drug delivery for lung targeting Image: 1. Lung: With metastatic melanoma, 2.Lung Histology: A clump of melanotic brown coloured (Arrow) cell H&E stain (5x), 3. A clump of alveoli cells and tumor cells with melanin pigment (Arrow) and few RBC Giemsa stain (100x), 4. Scintigraphy of rabbit image showing uptake of radiolabeled Nano formulation at 0 min and 3 hrs.