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Development of rat as an animal model to assess drug induced cardiotoxicity by Gallium-67

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Abstract

^{67}Gallium Citrate (^{67}Ga), an iron analogue radionuclide is commonly used compound in nuclear medicine for various diagnostic and therapeutic procedures as well as in experimental studies. Uptake of ^{67}Ga is having a complex mechanism with variable results. Present study is aimed to standardize and report the biodistribution pattern of ^{67}Ga in healthy Wistar rats and to establish uptake data for various organs and tissues. The adult Wistar rats of either sex were injected with ^{67}Ga-citrate and sacrificed 72 hours post injection. The weight and activity concentrations in different organs of interest and tissues were recorded. These results were decay corrected and these decay corrected results were used to calculate per cent injected dose per gram of organ/ tissue (%ID/g) and their ratio with each other. The ^{67}Ga activity (%ID/g) was found accumulated very high in spleen followed by liver, femur, kidney and lungs as compared with %ID/g of the whole animal.

Keywords: ^{67} Gallium Citrate, Wistar rats, Animal Model, Radioactive uptake

Radioisotopes are being used for the diagnosis and treatment of numerous clinical conditions for decades. ^{67}Ga is the radionuclide most widely used for the diagnosis of infections and malignancies viz. lymphoma, melanoma, lung cancer, myeloma, mesothelioma, testicular tumours, hepatoma and bone sarcoma, (Hayes 1978). However, since its discovery, the main interest in the diagnostic use of ^{67}Ga has been for Hodgkin's disease and Non-Hodgkin's lymphoma. Tumour models have demonstrated that the radiotracer to be an indicator of tumour viability, i.e., ^{67}Ga uptake is seen only in viable lymphoma tissue and not in fibrotic or necrotic tissue (Draisma *et al.,* 1998). At present, ^{67}Ga is viewed as one of the most dependable specialists for tumour imaging in lymphoma patients. Though ^{67}Ga scan has gained its importance in the management of lymphoma patients, the fact is that the agent is a nonspecific tumour tracer that localizes at the sites of inflammation as well (Line, 1991).

The mechanisms of ^{67}Ga uptake are still not completely understood. Research findings indicates that ^{67}Ga uptake displays the metabolic activity of tumor and its viability. Animal experiments' have confirmed that ^{67}Ga uptake is connected to cell proliferation and ribosomal protein synthesis. After intra venous injection of carrier-free ^{67}Ga, more than 90% is bound in plasma, mainly to transferrin. As ^{67}Ga is an iron analogue, it is commonly accepted that it is able to be replaced by some cations with a similar ionic radius (0.62 Angstrom), for example Fe^{+++} or Mg^{+++}. Consequently; ^{67}Ga gets approach to the cells via transferrin receptors. It is recognized that the cell surfaces in lymphomas are abundant in such receptors. The fact that gallium can bind to transferrin and enter tumour cells as a Ga-transferrin complex, is well documented. ^{67}Ga uptake in cultured tumour cells correlates with the concentration of transferrin.

Animal models are often used to test new radiopharmaceuticals for the treatment of tumour diseases, including both mice and rats. However, larger size of organs in rats than size of organs in mice makes sampling easier results in accepting the rat as an animal model (Spetz *et al.*, 2013). Also, the researchers suggested rat as a most suitable model to study the drug induced cardiotoxicities studies. It has also having additional benefit of having doxorubicin threshold values closest to the human values (Podesta *et al.*, 1994). This is an important area of research to improve already existing and new treatment methods, especially for disseminated tumour disease, when using radioactive substances labelled with ^{67}Ga. Commonly, a small amount of $^{67}Ga^{-}$ or free ^{67}Ga is released from the radiopharmaceutical *in vivo*. It is then important to know the biodistribution and the absorbed dose to normal tissues for ^{67}Ga and to determine potential risk organs when using such radiopharmaceuticals for treatment. To our knowledge, very limited physiological biodistribution

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data of ^{67}Ga is available. The uptake of ^{67}Ga is complex and influenced by various normal variants which produce variable findings of the uptake of ^{67}Ga in normal tissues.

Now a day's, Positron Emission Tomography (PET) by using ^{18}F Fluorodeoxyglucose (FDG-PET) is gaining importance due to encouraging initial reports yet ^{67}Ga-citrate has its importance as the scarcity of the well-equipped PET centres, high cost of scanning as well as the complex procedure. Therefore, present study intended to elucidate the physiologic phenomenon and to describe the pattern of its uptake as a potential pitfall in the evaluation of ^{67}Ga biodistribution and uptake studies. The purpose of this study was to develop rat as an animal model to study the uptake of Gallium-67 in Doxorubicin (DXR) induced cardiotoxicity.

Materials and Methods

Animal model

The studies were approved by the Institutional Animal Ethics committee of Mumbai Veterinary College, Mumbai vide No. MVC/IAEC/35/2015, dated 17.10.2015. The study was conducted on seven (07) healthy Wistar rats. These rats were weighing 180 – 250 grams with either sex (03 females and 04 males). The rats were kept separately as per sex in cages. These experimental animals were given free access to the clean drinking water and ad lib autoclaved food for 7 days, before injection of ^{67}Ga.

Radionuclides

^{67}Ga has been used for bio-distribution studies, which was manufactured by Isorad Ltd., Yavne, Israel. This radionuclide was imported and supplied by Saxsons Biotech Privet Limited, New Delhi as Gallium citrate.

Administration Gallium citrate

0.3 ml of radioactive material was administered via tail vein in each animal by using 1 ml insulin syringe. The syringe containing radioactive material was counted for radioactivity by using dose-calibrator (CRC-15R of Capintec, New Jersey) and immediately injected in animals without wasting any further time to avoid the reduction in the activity due to radioactive decay. The exact time of the injection was recorded and the syringe was again counted after injection to calculate the injected activity. The injected activity was calculated by using following formula:

Injected Activity = Activity in syringe after injection - Initial activity before injection.

Biodistribution

The rats were immobilized 72 hours post injection of ^{67}Ga under general anaesthesia with Isoflurane. The radioactivity count of the animal was recorded by dose calibrator and was immediately dissected and visceral organs viz. liver, kidneys, spleen, lungs, gut and femur were collected. The organs were weighed by an electronic weighing machine. These organs then washed with physiological saline and pat dried by using a clean muslin cloth. The radioactivity count and time of count were recorded for each of the organ. The residual carcass was wrapped and sealed in a polythene bag and radioactivity measured by dose calibrator. The background count was also recorded before every count for recording corrected count.

All the radioactive concentrations were corrected by subtracting the respective background count from the count of each organ. This corrected count was further decay corrected with respect to the time of injection. The results were calculated and expressed as a percentage of injected radioactivity dose per gram of organ (%ID/g). The ratio of %ID/g each measured organs to other organs was also calculated. The activity concentration in various organs at the time t (t=0), $C_{tissue}(t)$ as %ID/g was calculated by using formula as.

$$C_{tissue}(t) = \frac{A_{tissue}(t)}{A_{inj} \times m_{tissue}} \times 100\%$$

Where $A_{tissue}(t)$ is the activity in the sample at time t corrected for radioactive decay to t = 0, A_{inj} is the activity injected at time t = 0, and m_{tissue} is the mass of the tissue sample.

Statistical analysis

The statistical analysis of recorded data was undertaken as per methods outlined by Snedecor and Cochran (1994).

Results and Discussion

The decay corrected values of radioactivity tracers concentration in selected organs to the injection time point is expressed as %ID/g and their ratio with respect to each other is shown in Table No. 1 and Figure 1 and Table No. 2 and Figure 2 respectively.

Table 1: The Activity Concentration [$C_{tissue}(t)$], %ID/g (mean ± SE), of ^{67}Ga Citrate in Rats (n= 7) at 72 hours after injection

Tissue	Radioactivity uptake (%ID/g) (Mean ± SE)
Whole Animal	0.44 ± 0.03
Heart	0.56 ± 0.05
Liver	2.74 ± 0.13
Kidney	2.13 ± 0.21
Spleen	5.13 ± 0.45
Lungs	1.20 ± 0.19
Femur	2.61 ± 0.23
Gut	0.44 ± 0.07
Residual carcass	0.32 ± 0.04

The data are corrected for physical decay.

The mean uptake values for ^{67}Ga demonstrated that the whole animal ^{67}Ga uptake as 0.44 ± 0.03 %ID/g, which is lowest in mean %ID/g values of all the collected organs except the residual carcass. This gives an idea that the ^{67}Ga is having an affinity towards the visceral organs. The highest mean absorbed dose of ^{67}Ga was recorded in spleen i.e. 5.13 ± 0.45 %ID/g, followed by liver 2.74 ± 0.13 %ID/g, femur 2.61 ± 0.23 %ID/g, and kidney 2.13 ± 0.21 %ID/g, respectively. This may be because the Iron is mostly stored and/ or utilized by these organs. The iron in the form of ferritin and hemosiderin is present in the liver, spleen, and bone marrow (Hiroshi, 2014). Also some authors suggested that tissue-bound ^{67}Ga associates with iron-binding proteins such as transferrin, ferritin, and lactoferrin (Hartman and Hayes 1969; Gunasekera *et al.*, 1972; Hara 1974; Hegge *et al.*, 1977; Larson *et al.*, 1978 and 1979; Vallabhajosula *et al.*, 1980; Weiner *et al.*, 1983). The lungs also showed a considerable amount of radioactivity concentration of 1.20 ± 0.19 %ID/g. Heart followed by gut shows slightly higher activity than the values in whole body i.e 0.56 ± 0.05 and 0.44 ± 0.07, respectively. But, the concentration of activity in the residual carcass (0.32 ± 0.04 %ID/g) was lower than that of the whole animal concentration. This lower concentration is logically justified as the organs with higher concentrations were removed for individual study.

The ratio of activity present in different organs (mean %ID/g) was calculated and presented in Table No. 2. We tried to develop rat as an animal model for the cardiotoxicity studies induced by Doxorubicin. Hence the organ of interest i.e. heart shown highest ratio (%ID/g) with residual carcass (1.85 ± 0.23) followed by gut (1.38 ± 0.18), whole animal (1.30 ± 0.15) and lungs (0.52 ± 0.07) respectively. Moreover, heart showed lowest ratio (%ID/g) with kidneys (0.27 ± 0.03), femur (0.23 ± 0.04), liver (0.21 ± 0.03) and spleen (0.12 ± 0.02) respectively.

Likewise, the ratio of mean %ID/g of collected organs with heart was also calculated and having highest ratio of 9.64 ± 1.15 with spleen followed by liver 5.21 ± 0.62; femur 4.91 ± 0.66; kidney 3.88 ± 0.39 and lungs 2.26 ± 0.47. Whereas, the heart demonstrated the lowest ratio of 0.85 ± 0.18, 0.83 ± 0.09 and 0.59 ± 0.07 with gut, whole animal and residual carcass, respectively.

The diverse studies shows that the ^{67}Ga having affinity towards inflamed tissues. The gallium has proved its reliability for evaluation of myocardial (Beuscari *et al.*, 1987) and pericardial (Huikuri *et al.*, 1988) inflammation and superior over endomyocardial biopsy (O'Connell *et al.*, 1984).

To study the human cardiovascular diseases, the ideal animal model should have five characteristics: (i)

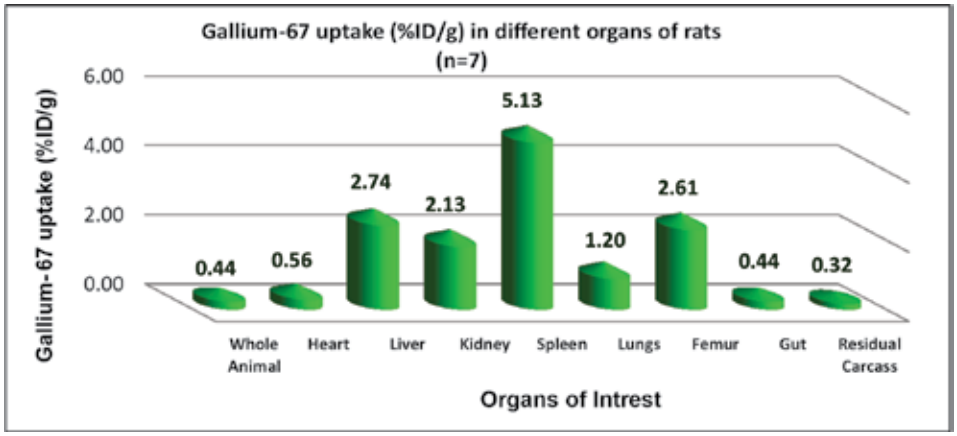

Fig. 1: ^{67}Gauptake (%ID/g) in different organs of rats (n=7)

Table 2: Ratio (Mean ±SE) of %ID/g of collected organs with %ID/g of other organs

Ratio of organ → / ↓Ratio with organ	Whole Animal	Heart	Liver	Kidney	Spleen	Lungs	Femur	Gut	Residual carcass
Whole Animal	--	1.30 ± 0.15	6.37 ± 0.56	4.82 ± 0.41	12.07 ± 1.46	2.71 ± 0.35	6.21 ± 0.82	1.01 ± 0.11	0.72 ± 0.04
Heart	0.83 ± 0.09	--	5.21 ± 0.62	3.88 ± 0.39	9.64 ± 1.15	2.26 ± 0.47	4.91 ± 0.66	0.85 ± 0.18	0.59 ± 0.07
Liver	0.16 ± 0.01	0.21 ± 0.03	--	0.78 ± 0.07	1.89 ± 0.17	0.44 ± 0.07	0.97 ± 0.11	0.16 ± 0.02	0.12 ± 0.01
Kidney	0.22 ± 0.02	0.27 ± 0.03	1.37 ± 0.15	--	2.59 ± 0.35	0.56 ± 0.06	1.34 ± 0.22	0.22 ± 0.03	0.15 ± 0.01
Spleen	0.09 ± 0.01	0.12 ± 0.02	0.56 ± 0.06	0.44 ± 0.07	--	0.24 ± 0.04	0.54 ± 0.07	0.09 ± 0.02	0.07 ± 0.02
Lungs	0.41 ± 0.05	0.52 ± 0.07	2.56 ± 0.33	1.90 ± 0.21	4.73 ± 0.63	--	2.48 ± 0.41	0.38 ± 0.02	0.30 ± 0.05
Femur	0.19 ± 0.04	0.23 ± 0.04	1.14 ± 0.19	0.90 ± 0.18	2.17 ± 0.45	0.50 ± 0.10	--	0.18 ± 0.03	0.14 ± 0.03
Gut	1.06 ± 0.11	1.38 ± 0.18	6.66 ± 0.66	5.08 ± 0.61	12.5 1 ± 1.52	2.68 ± 0.17	6.36 ± 0.81	--	0.78 ± 0.12
Residual carcass	1.42 ± 0.09	1.85 ± 0.23	9.24 ± 1.22	6.79 ± 0.60	17.68 ± 2.70	3.86 ± 0.52	9.08 ± 1.50	1.44 ± 0.18	--

able to mimic the human disease, (ii) allow studies in chronic, stable disease, (iii) produce symptoms which are predictable and controllable, (iv) satisfy economical, technical and animal welfare considerations, and (v) allow measurement of relevant cardiac, biochemical and haemodynamic parameters. No model exactly mimics the human diseases bur rat model is appropriate model for DXR induced cardiotoxicity studies. This model is having main advantage that it is technically simple, non-invasive, economical and has a short time course (Doggrell and Brown, 1998).

The rat has been established as an accurate, reproducible and cost effective animal model for conducting DXR induced cardiotoxicity studies (Mettler *et al.*, 1977 and Zbinden *et al.*, (1978). Also, the rat is having the lowest threshold of irreversibility for DXR induced cardiotoxicity and closest to the human threshold values. Hence the extrapolation of results from studies in rats is substantial for humans (Podesta *et al.,* 1994). Galdhar (2008) developed rabbit as an animal model for DXR induced toxicity by gallium scintigraphy. In his dissertation, he concluded that analysis of total count and average count heart to liver ratio at 48 hrs in anterior chest images were suitable parameters to calculate the healthy (non inflammatory) status of heart.

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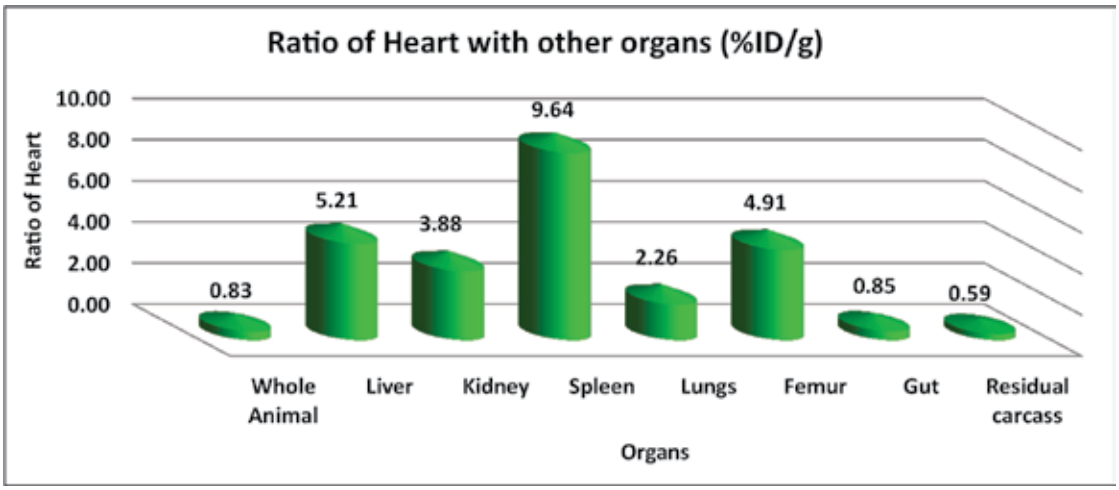

Fig. 2: Ratio of activity concentration in heart with activity concentration in different organs.

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