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Studies on the Disposition of Technetium-99m Labeled Foot-and-mouth Disease Vaccine in Guinea Pigs

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ABSTRACT

Background: Foot-and-mouth disease (FMD) is a widespread and contagious disease affecting various animal species. The effectiveness of control tools is impeded by the high costs associated with animal experimentation, an incomplete understanding of host immune systems and a scarcity of immunological reagents. 99mTc radiopharmaceuticals, known for their versatile chemistry, play a crucial role in preclinical research and drug development. The objective of the present study was to develop nuclear medicine-enabled strategies for custom designing and uplifting engineered vaccines, specifically nanoparticle vaccines, in guinea pigs.

Methods: In the present study, we elucidate the technique used for direct radiolabelling of the FMD vaccine and its subsequent distribution within guinea pigs. The study involved the evaluation of 99mTc-radiolabeling and quality control, as well as the disposition of the 99mTc-FMD vaccine in guinea pigs.

Result: Radiolabelling and stability studies showed 95.00% efficiency and 92.93% stability. The study demonstrated that the 99mTc-FMD vaccine showed increased uptake and delayed clearance from lymphoid organs, particularly the spleen and prescapular lymph node, compared to plain 99mTc.

Key words: 99mTc radiopharmaceuticals, Foot-and-mouth disease (FMD) vaccine, Guinea pigs, Lymphoid uptake, Vaccine development.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious and widespread disease that infects a range of cloven-footed animal species, causing acute lesions in the tongue, snout, buccal cavity, feet and teats (Anonymous, 2023). Its economic impact and rapid dissemination render it a dreaded livestock disease. The development of effective tools for FMD control has been hindered by factors such as, exorbitant costs associated with animal experimentation, gaps in our understanding of host immune systems and a shortage of immunological resources (Radostits *et al*., 2007). Technetium-99m (99mTc) radiopharmaceuticals are single isotopes used in over 80% of diagnostic procedures due to their availability from the 99Mo/99mTc generator. Their versatile chemistry allows for the production of various complexes with specific characteristics, making them useful for radiopharmaceutical development. Earlier, Galdhar *et al.* (2010); D'Souza *et al*. (2013); Nagarsekar *et al*. (2014); Galdhar *et al.* (2022) and Galdhar *et al.* (2024) successfully used 99mTc-radiopharmaceuticals and I-125 in preclinical and clinical research for novel drug development and endocrinological diagnosis in veterinary practice.

The objective of this study was to devise novel strategies within the realm of nuclear medicine to facilitate the tailored design and enhancement of engineered vaccines, with a specific focus on nanoparticle vaccines. In this paper, we elucidate the technique used for radiolabeling the FMD vaccine and its subsequent distribution within guinea pigs.

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MATERIALS AND METHODS

Experimental animals and statutory approval

The present study was undertaken at the Department of Veterinary Nuclear Medicine including Radio Isotope Laboratory, Mumbai Veterinary College, Maharashtra Animal and Fishery Sciences University, Parel, Mumbai (India). This radiation facility is approved as a research facility for veterinary use by the Atomic Energy Regulatory Board (AERB), Govt. of India. The studies were approved by the Institutional Animal Ethics Committee (IAEC) and

Institutional Bio-Safety Committee (IBSC) of Mumbai Veterinary College, Mumbai; before its commencement. The study was conducted on three (03) healthy guinea pigs. These guinea pigs weighed 350-450 grams with either sex. These animals were given free access to clean drinking water and *adlib* food for 7 days to acclimatize, before injection of 99mTc-FMDV vaccine.

99mTc-radiolabeling and quality control assessment

Radioactive 99mTc was obtained by elution from the parent element molybdenum (99Mo) by alumina column solvent extraction. Conventional oil adjuvanted foot-and-mouth disease vaccine was procured from the market to undertake radiolabelling, stability and 99mTc uptake studies. The vaccine was radiolabelled using the direct radiolabelling method, with slight modifications, as described by Rhodes *et al.* (1986). Briefly, autoclaved vials (10 ml volume) were purged with Nitrogen, 1 ml of the conventional vaccine was added to 10 ml vial, followed by the addition of 100 µl of stannous chloride (2 mg/ml, 0.1 M HCl) under nitrogen atmosphere and incubated for 21 hours. Freshly eluted 1 ml of 99mTc Pertechnetate (4 mCi) was added in the same vial and mixed well while maintaining the nitrogen atmosphere. pH was adjusted to 6.5 to 7.0 by adding 0.1 M NaOH. The reaction mixture was gently stirred and incubated at room temperature for 30 minutes under slow orbital shaking at 150 rpm. The vaccine was labeled following all standard recommended aseptic and radiation safety procedures. After radiolabeling, the radiolabelled product was tested for radiolabelling efficiency using thin-layer chromatography (TLC). Radiolabeling efficiency was assessed by applying 5 µL of the labeled formulation at a 1.5 cm distance from the base of the strip and allowing it to migrate to the solvent front. TLC studies were conducted using silica gel-coated strips and normal saline as a mobile phase. Radioactivity was quantified by cutting the strip in two parts, in the proportion of 65% upper and 35% lower part being used for measurement. Free 99mTcO4 migrated with a normal saline to the strip's solvent front, while the radiolabelled formulation was retained at the application point. The percent of radioactivity at the origin was calculated as 100 × [CPM at origin/(CPM at origin + CPM at solvent front)]. The stability studies involved centrifuging a cattle blood sample, adding 300 µl of serum and radiolabelled product, shaking the tube and maintaining it at ambient temperature. The stability of the radiolabelled product was measured at 1, 3 and 6 hrs, respectively.

99mTc-FMD vaccine disposition studies

The disposition studies were performed by injecting 0.5 ml 99m Tc-FMD vaccine intramuscularly in guinea pigs. At time intervals of 1, 3 and 6 hrs post-injection, one guinea pig was sacrificed in a CO_2 chamber and the organs of interest were collected. The background count was recorded before each count to compute a corrected count. Radioactive concentrations were corrected by subtracting the background count from each organ count and decay was corrected with respect to the injection time. Results were expressed as a percentage of injected radioactivity dose per gram of organ (%ID/gm).

For comparison and to appraise the radioactive uptake pattern of 99mTc-FMDV vaccine, the same procedure was undertaken in 3 guinea pigs with plain $99mTcO_4$.

RESULTS AND DISCUSSION

Radiolabelling and stability studies showed 95.00% efficiency and 92.93% stability in the presence of serum for six hours with a conventional FMD vaccine. Results of comparative uptake of 99mTc and 99mTc-FMD vaccine uptake over a time period of 6 hrs are presented in Table 1. At 1 hr, the uptake (% ID/gm) of 99mTc and 99mTc-FMD vaccine for thyroid, lungs, kidneys, liver, spleen and prescapular lymph node was 0.74 and 0.08, 0.43 and 0.41, 0.82 and 2.13, 0.60 and 0.56, 0.30 and 0.10; and 0.11 and 0.29 %, respectively. At 3 hrs, the uptake (% ID/gm) of 99mTc and 99mTc-FMD vaccine for thyroid, lungs, kidneys, liver, spleen and prescapular lymph node was 6.40 and 0.44,

Table 1: 99mTc and 99mTc- FMD vaccine uptake (%ID/g) in guinea pigs over a period of time.

Organ	Parameters	1 hrs		3 hrs		6 hrs	
		99mTc	99mTc- FMDV	99mTc	99mTc- FMDV	99mTc	99mTc- FMDV
Thyroid	Uptake (%ID/g)	0.74	0.08	6.40	0.44	0.50	0.18
	99mTc- FMDV/99mTc		0.10		0.06		0.36
Lungs	Uptake (%ID/g)	0.43	0.41	0.13	0.09	0.12	0.08
	99mTc- FMDV/99mTc		0.95		0.69		0.66
Kidneys	Uptake (%ID/g)	0.82	2.13	0.35	8.8	0.21	2.15
	99mTc- FMDV/99mTc		2.59		25.14		10.23
Liver	Uptake (%ID/g)	0.60	0.56	0.18	1.20	0.25	0.41
	99mTc- FMDV/99mTc		0.93		6.66		1.64
Spleen	Uptake (%ID/g)	0.30	0.10	0.13	0.36	0.08	0.22
	99mTc- FMDV/99mTc		0.33		2.76		2.75
Prescapular lymph node	Uptake (%ID/g)	0.11	0.29	0.08	2.27	0.06	0.10
	99mTc- FMDV/99mTc		2.63		28.37		1.66

0.13 and 0.09, 0.35 and 8.80, 0.18 and 1.20, 0.13 and 0.36; and 0.08 and 2.27%, respectively. At 6 hrs, the uptake (% ID/gm) of 99mTc and 99mTc-FMD vaccine for thyroid, lungs, kidneys, liver, spleen and prescapular lymph node was 0.50 and 0.18, 0.12 and 0.08, 0.21 and 2.15, 0.25 and 0.41, 0.08 and 0.22; and 0.06 and 0.10%, respectively. Table 1 displays the localization of 99mTc-FMD vaccine uptake in guinea pigs over time, based on disposition pattern. The study found that the 99mTc-FMD vaccine showed increased uptake and delayed clearance from lymphoid organs, particularly the spleen and prescapular lymph node, compared to plain 99mTc. Uptake was also noticed in the liver at 1, 3 and 6 hrs with delayed clearance. The study indicates that the 99mTc-FMD vaccine formulation remains stable until 6 hrs due to minimal uptake at the thyroid. The kidneys showed the highest radioactivity levels at 3 hrs, which cleared up to 60% by 6 hrs, indicating the presence of 99mTc-FMD vaccines in circulation.

In the present study, it is observed that the 99mTc-FMD vaccine showed increased uptake and delayed clearance from lymphoid organs, especially the spleen and prescapular lymph node as compared to plain 99mTc. Thus, the comparative uptake of 99mTc and 99mTc-FMD vaccine demonstrated that the latter has considerable uptake in lymphoid organs. These findings are in close agreement with the earlier findings (Doel 1996; Langellotti *et al*., 2012 and Carr *et al.,* 2013). They reported that FMD vaccination triggers rapid T-cell responses in cattle showing CD4+ T-cell proliferation as early as 7 days post-vaccination. Further, the inactivated FMD virus increases CD8+ and regulatory T-cell numbers in the spleen. In our study, we have not navigated the immune response caused by FMD vaccination, but the present study confirms the hypothesis that the FMD vaccine has been entrapped by lymphoid organs.

CONCLUSION

The study highlights the process of radiolabeling and examines the uptake of a conventional foot-and-mouth disease vaccine in the lymphoid organs of guinea pigs. This suggests the feasibility of radiolabeling various vaccine formulations, allowing for the exploration of antigen uptake. Such investigations provide valuable insights into the fate of the vaccine upon inoculation and its immunogenic potential.

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Conflict of interest

The authors declare that there is no conflict of interest.

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