Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Feasibility of ^{99m}Tc-Phytate as a Lower Lymphoscintigraphic Agent in Dogs

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ARTICLE HISTORY (16-205)

Received: August 07, 2016
Revised: April 09, 2017
Accepted: April 12, 2017
Published online: May 18, 2017

Key words:
^{99m}Tc-phytate
Dog
Lymphoscintigraphy
Nuclear medicine
Radiopharmaceutical

A B S T R A C T

The purpose of this study was to evaluate availabilities of nuclear medicine images that could replace lower lymphangiography using conventional radio-pharmaceutical. Six beagles were performed lymphoscintigraphy after injected with 300 microcurie of ^{99m}Tc-phytate in 0.2ml, widely used in human medicine subcutaneously on 1st and 2nd interdigital space each of hind legs. To get images of popliteal lymph node, inguinal lymph node, iliac lymph node and thoracic duct, animals were laid supine beneath a gamma camera, and images at 0, 30, 60 and 120 min were obtained. The uptake rate of popliteal lymph node, bladder, and liver was measured in acquisitioned image. In this study, the image of popliteal lymph node is simply like that of a human, but it was not available for other lymph nodes above popliteal lymph node. Voluntary exercise or massage was not performed in anesthesia condition and that could be the reason for limitation in upper moving of radiopharmaceuticals. In conclusion, ^{99m}Tc-phytate has a limitation for lower lymphoscintigraphy in dogs.

To Cite This Article: Cho Y and Lee K, 2017. Feasibility of ^{99m}Tc-Phytate as a lower lymphoscintigraphic agent in dogs. Pak Vet J, 37(3): 331-334.

INTRODUCTION

Lymphoscintigraphy is evaluating the uptake of a radiopharmaceutical that is injected into the periphery and moved into the regional lymph nodes (Szuba *et al.*, 2003). This skill might both determine the cause of swelling and its pathophysiology (Ter *et al.*, 1993; Dabrowski *et al.*, 2008). Lymphatic flow occurs by the unique contraction of lymph and the force of external tissues (Bellini and Hennekam, 2014). Lymphedema is caused by the accumulation of lymph and develops due to damage and deformity of the lymphatic system. Lymphedema is characterized by four pathologic features: edema, chronic inflammation, excess tissue protein (Mortimer and Levick, 2004; Burnand *et al.*, 2012). The face, neck, and abdomen are affected, but it affects the extremities in general (Szuba *et al.*, 2003; Warren *et al.*, 2007).

As a chronic debilitating disease, lymphedema is often observed. Early diagnosis and treatment are very important in reducing the risk of progression of the disease and its complications (Kumar *et al.*, 2003). Detections for lymphatic insufficiency include absence or delay of lymphatic transport from injection site, the presence of radiotracer uptake in dermal lymphatics (Dylke *et al.*, 2013).

Secondary lymphedema is mostly caused by cancer or damage to the lymphatic system due to treatment (Suehiro *et al.*, 2014). As the lymphatic system approaches various tissues of the body, it leads to the major progression of cancer cell transportation and to major transportation causing metastasis (Zwaans and Bielenberg, 2007). If cancer cells are not removed during the process of capturing and killing cancer cells in the intervening lymph nodes, metastasis to another site occurs (Sumner *et al.*, 2002).

To diagnose lymphatic system disorders, lymphangiography using contrast media has been performed to date (Dinh *et al.*, 2016). It difficult to examine the exact lymphatic flow of the primary tumor site of malignant melanoma and the lymphatic flow of parasternal lymphatics in breast carcinoma. Additionally, lymphangiography wherein examination is done by direct surgical incision on the lymphatic vessel and by injecting oil-based contrast material has been recently replaced by lymphoscintigraphy in nuclear medicine. It is non-invasive and allows for quantitative assessment of lymphatic flow and dynamic test (Lam *et al.*, 2009; Karacavus *et al.*, 2015).

Radiopharmaceuticals primarily used in lympho-scintigraphy include ^{99m}Tc-tin colloid and ^{99m}Tc-phytate.

These two radiopharmaceuticals move to the lymph node through lymphatic vessels after subcutaneous injection (Seyhan *et al*., 2015). Phagocytosis by the macrophage provides time of lymphatic flow and morphological imaging of lymph (Witte *et al*., 2000; Hatta *et al*., 2005). It is used for assessing lymph duct atresia and primary and secondary lymphedema (Burnand *et al*., 2012; Karacavus *et al*., 2014). ^{99m}Tc-phytate used in this study is a radionuclide, which is the most commonly used for humans, and phagocytosis of the reticuloendothelial system was used.

The purpose of this study is to evaluate the usefulness of lymphoscintigraphy in beagles, as an alternative for lower lymphangiography that uses a conventional staining reagent, is invasive, and poses difficulties in conducting dynamic and quantitative tests. In contrast, lymphoscintigraphy is noninvasive and can facilitate dynamic and quantitative assessments.

MATERIALS AND METHODS

This study was approved by Committee on Ethics for Experimental Animal of College of Veterinary of Chonbuk University. Six adult purpose-bred dogs weighing 10.0±0.3kg (mean±SD) and ranging in age from 1 - 5 years were supplied by the laboratory animal facilities at the Veterinary Medicine Clinic of Chonbuk University. The dogs were deemed normal based on the results of physical examination, routine hematology and clinical chemistry determinations, thoracic and abdominal radiographs, and abdominal ultrasound.

Lymphoscintigraphy is using gamma camera (E.CAM, Siemens, Germany) in nuclear medicine Department under Kon kuk university hospital. Dogs were fasted for 24 hrs before the lymphoscintigraphy scan in preparation for general anesthesia and injected 0.04ml/kg medetomidine on gluteal muscle by intramuscular injection.

To study each group of lymph nodes, after radioisotope ^{99m}Tc and commercial phytate mix, 300 microcurie of this preparation was injected subcutaneously into the dorsum of the interdigital 1, 2 and 2, 3.

Six dogs were used for this study, and 12 sites in all were injected. After phytate injection, whole body scanning was performed from radiopharmaceuticals injected areas to upper direction with 10cm/60sec speed.

We detected anterior and posterior whole body image post radioisotope injection at 0 min, 30 min, 60 min and 120 min. The sites of injection were monitored by a scintillation probe attached to a scaler, and counts obtained for 120 min were used to calculate the clearance rates. The images were obtained with a scintillation camera. Low-energy all-purpose collimators were used for ^{99m}Tc-phytate scintigraphy, window is 15%, center line is 555-565 (^{99m}Tc- autopeak), matrix size is whole body, magnification is 1.00, patient position is supine, data processing procedure is mirage processing, prism processing is used, film is used codonics NP-1660MP.

Count rate was measured by drawing each ROI such as left, right interdigital ROI, popliteal lymph node ROI, bladder ROI, liver ROI during image processing at 0 min, 30 min, 60 min and 120 min.

RESULTS

Within minutes after subcutaneous administration of ^{99m}Tc-phytate, there was evidence of transfer of activity to the rest of the body. The draining lymph nodes began to appear on the scan several minutes after injection (Fig. 1). Early visualization of the bladder occurred in 5 min (Fig. 2). The activity in the bladder appeared more intense (Fig. 3), and the liver was visualized within 120 minutes after administration of the agent (Fig. 4). During the first 30 min there was some background activity, which increased significantly.

Count rate of left interdigit at 0 min and 120 min was decreased by 82.3% from average 615.20 to 475.70. Count rate of right interdigit at 0 and 120 min also decreased by 94.1% from average 615.70 to 579.90. Count rate changes of right and left interdigit at 120 min showed that agent was drained faster by 11.8% on left interdigit than right interdigit (Table 1).

Count rate of left popliteal lymph node, right popliteal lymph node and bladder at 0 min and 120 min was increased. It was increased by 195.4% from average 44.72 to 86.2 1 on left popliteal lymph node, 183% from 60.50 to 110.00 on right popliteal lymph node and 127% from 4.60 to 5.87 on bladder.

The result of count rate of left popliteal lymph node and right popliteal lymph node at 120 min showed that the phytate uptake of left popliteal lymph node was stayed 12.4% longer than right popliteal lymph node. Both popliteal lymph node of 2nd dog among six dogs were observed 60 min after injection, and liver was showed 120 min after injection.

DISCUSSION

In this study, we studied diagnostic possibility in lower lymphoscintigraphy and examination on utility by using ^{99m}Tc-phytate which was presently reused. ^{99m}Tc-phytate is known to show better uptake in liver than spleen when injected into blood vessel forming particle of very small diameter in blood vessel, however, when putting more calcium into pre-injection to form large particle, the uptake of spleen relatively increases. Though it was not an image for sentinel lymph node, but ^{99m}Tc-phytate has been used in lymphoscintigraphy since long (Moslehi *et al*., 2015). ^{99m}Tc-phytate is known to be a particle of 100-200 nm diameter and is also known to show 8 nm diameter when there is no binding with calcium through electronic microscope like this, the size of phytate and formation of colloid are up to the existence of calcium (Ravari *et al*., 2015).

The phytate went up faster in left interdigit than in right interdigit, but considering the result of 120 min coefficient rate, the thing that more amount stays in left popliteal lymph node means that the movement in lymph duct between left interdigit and popliteal lymph node is fast but in the popliteal lymph node, the upward lymph duct flow gets more restriction than right.

It remains to be studied whether it is related with the intramuscular injection of anesthetic drug into right gluteal muscle. The uptake in bladder is seen from 30 min and what is observed in liver at 120 min image is not the metabolism of ^{99m}Tc-phytate collected in the bladder but seems to be the uptake of phytate and free ^{99m}Tc of unbound ^{99m}Tc.

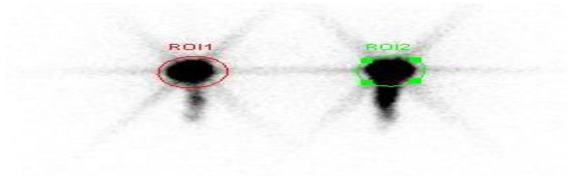

Fig. 1: A scintigram at the site of injection and subcutaneous injection show good concentration of ^{99m}Tc-phytate (ROI 1-left, ROI 2-right).

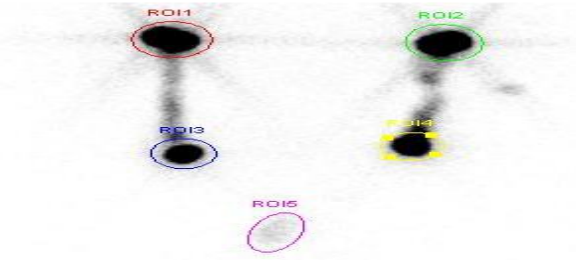

Fig. 2: A scintigram obtained 30 min after subcutaneous injection of ^{99m}Tc-phytate into a dog demonstrate good concentrations in popliteal lymph nodes (ROI 1-Lt interdigit, ROI 2- Rt interdigit, ROI 3- Lt popliteal lymph node, ROI 4- Rt popliteal lymph node, ROI 5- bladder).

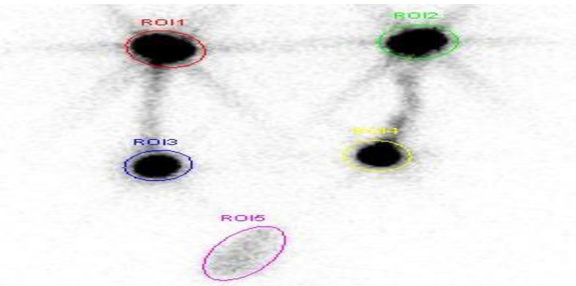

Fig. 3: Region of interest(ROI) at a scintigram obtained from a dog 60 min after subcutaneous injection of ^{99m}Tc-phytate (ROI 1- Lt interdigit, ROI 2- Rt interdigit, ROI 3- Lt popliteal lymphnode, ROI 4- Rt popliteal lymphnode, ROI 5- bladder).

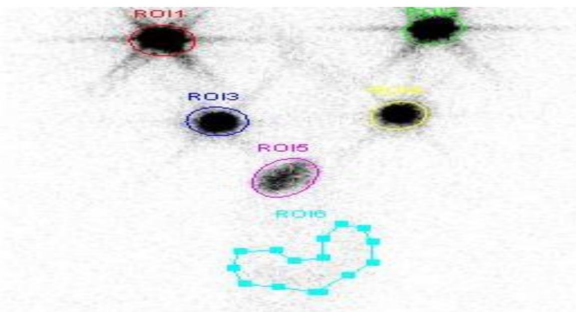

Fig. 4: ROI scintigram obtained from a dog 120 min after subcutaneous injection of ^{99m}Tc-phytate into dog demonstrates activity in liver, there is minimal visualization (ROI 1- Lt interdigit, ROI 2- Rt interdigit, ROI 3- Lt popliteal lymph node, ROI 4- Rt popliteal lymph node, RO I5- bladder, ROI 6- liver).

Table 1: The comparison of counts that uptake of ^{99m}Tc-phytate in 0, 30, 60 and 120 min of left interdigit, right interdigit, left popliteal, right popliteal, bladder, and liver

Organ	0 min	30 min	60 min	(Mean±SD) 120 min
a	577.9±175.1	561.9±195.6	537.3±162.2	475.7±170.1
b	615.7±145.5	583.0±91.3	620.9±102.2	579.9±122.9
c	53.7±46.6	109.8±56.5	94.4±43.7	86.22±45.9
d	72.6±55.2	138.4±69.2	163.7±135.2	110.04±52.3
e	0±0	4.6±2.5	4.9±1.6	5.9±2.6
f	0±0	0±0	0±0	1.1±0.2

A=Interdiz-left, b=Interdiz-right, c=Popliteal-left, d=Popliteal-right, e=Bladder, f=Liver.

As both interdigits show continuous decreasing following the time while both popliteal lymph nodes show continuous increasing until 60 min and turns to decreasing after 60 min, it turns out to get the most excellent data upon observing disorder of popliteal lymph node from the image at 60 min (Burnand *et al*., 2011; Kandeel *et al*., 2013).

To represent lymph node of groin-pevic- aortic region in humans, radio pharmaceuticals are administered then the patient is asked to walk or perform exercise of extremity to increase phytate and then perform massage for smooth upper moving of radiopharmaceuticals (Karavus *et al*., 2015). In this animal study, however, voluntary exercise or massage was not possible in anesthesia condition and that could be the reason for limitation in upper moving of radiopharmaceuticals.

The lymph can be circulated in any direction in the plexus of lymph of lymph capillary vessel, skin and subcutaneous texture. Such circulative direction is up to the strength of external force of lymph system. Also, the course is decided by the elements of gravity, passive movement, and massage. If activity of deep lymph gland is stimulated, then lymph node located in sacrum can be open. If the part can be flown by the massage or gravity, the lymph will be flown out through other passage by the direction of external force. It shows that lymph scarcely flows when the muscle doesn't move through a few animal experiments. The lymphocele increases when performing massage on the muscle by quoting the study on the effect of massage on the lymph circulation (Tartaglione *et al*., 2010; Kandeel *et al*., 2013).

Actually, there was hardly any lymph gland circulation in the part which didn't move. So it was thought to be desirable to raise the part on which the massage is performed for efficient circulation of lymph gland. Therefore, it is judged that omitting the massage from this experiment as a limitation would have influenced on the uptake of phytate, and so accurate massage is necessary for a long time.

Compared to human, it is judged that dog's lymph duct diameter, different lymph moving speed due to nonexistence of muscle contraction caused by anesthesia, and over growth of colloid by excessive binding of popliteal lymph node's calcium and phytate caused upper moving restricted.

Conclusions: From this experiment performed on the dog through Tc-99m phytate test, continuous and clear uptake was seen for 2 hours of scanning from injection site, interdigital to popliteal lymph node; however, it was hard to observe lymph duct and lymph node other than popliteal lymph node. In this study, the weight-based amount of dose used for human was applied to the dog; as there was star-shaped artifact was seen in the injection site, interdigital, it is judged that there was negative effect in lymph duct observation between interdigital and popliteal lymph node. Therefore, additional study is required regarding the optimal dose for the dog and smooth lower lymph system flow through autonomous walking after injecting subdermal medicine into interdigital like the human case. In addition, different diameter of lymph duct with human, and particle size followed by the combination with phytate for calcium density inside lymph duct shall be studied.

After interdigital subdermal injection which was done on the dog for the first time, the lymphoscintigraphy using the only domestically permitted phytate in anesthesia state showed excellent image before popliteal lymph node but revealed a few limitations providing the basis for further study.

To obtain image, however, after performing subdermal injection and taking autonomous exercise more than an hour like human case, it would be useful for entire lower lymph system diagnosis like human case.

Authors contribution: KL contributed to design the entire experiment. YC executed the experiment. KL and YC analyzed the images, count rate, and statistical analysis. KL and YC are involved in discussing the contents of the manuscript and agreed to publication.

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