

# Study of the Dextran-DTPA-Gd at rabbit popliteal fossa lymph node metastasis from thigh transplanted tumor with interstitial magnetic resonance lymphography

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**Abstract Objective:** The aim of the study was to investigate the developing situation of the interstitial magnetic resonance (MR) lymphoid contrast agent Dextran-DTPA-Gd through the rabbit popliteal fossa lymph node metastasis from thigh VX2 transplanted tumor injection to show targeting enhanced metastatic lymph nodes and lymphatics. **Methods:** VX2 tumor was transplanted to the right hind limb quadriceps of 12 healthy New Zealand rabbits and the left side as a contrast. Eight rabbits had homonymy popliteal lymph node metastasis after 1 month through 3.0 GE MRI and they were later injected with lymphatic targeting contrast agent Dextran-DTPA-Gd 0.4 mL ( $3.96 \times 10^{-3}$  mol/L) through bilateral hindlimb toe web respectively. Enhanced MR images were obtained with interval 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, 40 min, 45 min, 50 min, 55 min, 60 min, 2 h, 4 h, and 24 h. The signal intensities before and after enhancing were measured to calculate the enhancing rates (E%) of popliteal lymph node and the popliteal lymph node signal intensity-time curves were drawn to observe the development of cancer metastasis lymph nodes and lymphatics and to compare the differences of interval sides. **Results:** Ten minutes after injected into the rabbit's bilateral hindlimb toe web, we could see hind lymphatic and popliteal lymph nodes were strengthened significantly and evenly without blood vessels developing. The signal reached a peak after 35 min with E% to 315%, which decreased to 205% after 4 h and would be undifferentiated with the surrounding tissues after 24 h. Statistical analysis was made to popliteal lymph node enhancement rate. It was considered statistically significant as long as  $P < 0.05$ . The tumor-side popliteal lymph node manifested as coarse and irregular shape, lymphatic vessels tortuous dilated and lymphatic chain incomplete as a result of tumor infection. **Conclusion:** Dextran-DTPA-Gd is specific to lymphoid tissue development. It can targeting display regional lymphatic drainage concretion and the morphology of normal and cancer cells metastasis lymph nodes rapidly.

**Key words** magnetic resonance (MR); lymphography; Dextran-DTPA-Gd; rabbits VX2 tumor; popliteal lymph nodes

Invasion and metastasis are not only the main features of malignant tumors, but also an important reason to affect efficacy and patients death. Lymph node metastases is the most important way to the initial stage tumor, which is also an important indicator to judge prognosis [1, 2]. Its treatment is mainly extended resection of the primary tumor and draining lymph node dissection which mainly due to tumor location, size, degree of differentiation and

lymph node size, CT or MR and other imaging performance. Therefore, it has important clinical significance to determine the occurrence of cervical lymph node metastasis.

Interstitial magnetic resonance lymphography (IM-RLG) is a method that inject contrast agent into the lymphatic capillaries via skin or mucous interstitial to show the lymphatic system through lymphatic drainage. It can clearly show the distribution of lymph, form of lymphatic and the lymph node morphology with MR scanning and three dimensional reconstruction of high resolution.

IMRLG contrast agents can be divided into small molecular forms and large molecular forms according to its molecular size. Small molecule formulations are mainly Omniscan (Gadodiamide), Magnevist (Gadopentetate Dimeglumine) and so on. They have been used to display the lymphatic vessels and lymph nodes of experimental animal limbs with IMRLG in recent years which have achieved good results and have continually been used for human upper limb lymphedema diagnosis [3-5]. Macromolecular IMRLG contrast agent is coupling large molecular aggregates and paramagnetic material to form a preparation with large molecular weight, not easy through the capillary membrane and only be selectively absorbed by lymphatic, such as albumin-gadolinium Diethylenetetramine pentaacetic acid (HSA-Gd-DTPA), parcel gadolinium liposome etc [6, 7]. However, because of its relatively large molecular mass, although it can be selectively absorbed by lymphatic vessels and lymph nodes, it still needs several hours to reach the enhancement MR peak, which as a result make its clinical applications limited.

This experiment developed a macromolecular contrast agent Dextran-DTPA-Gd based on differences in the link between lymphatic capillaries and capillary endothelial cells as compared to the previous experiment. It can provide a relevant theoretical basis for the next study of head and neck cancer by conduct lower limb lymphography on rabbit VX2 tumor model with popliteal lymph node metastasis.

## Materials and methods

### Experimental animal

Healthy clean New Zealand white rabbits, adult, male, body mass 2.7–3.7 kg, all provided by Animal Experimental Center, the Affiliated Hospital of Medical College of Qingdao University, China. The disposal of the animals during the experimental process fully obeyed the requirements of animal ethics.

### Experimental reagents

#### *Dextran-DTPA-Gd*

Condense the Dextran with diethylene triamine pentaacetic acid (DTPA) and then chelated to form strong paramagnetic agent gadolinium ion (Gd) to become polymer complexes Dextran-DTPA-Gd. The agent was produced by the Medicine College of Ocean University of China [8], with concentration  $3.96 \times 10^{-3}$  mol/L, molecular size of 130 nm on average, osmotic pressure of 77 mOsm/kg·H<sub>2</sub>O and the relative molecular mass of about 45 000 Da.

#### *Rabbit VX2 tumor tissue blocks*

Derived from epithelial malignant cells induced by Shope virus from papilloma, which was belonged to the squamous cell carcinoma (provided by the Pharmacy Department Laboratory of Ocean University of China).

The strain was built at 1940. It could be inoculated in rabbits and had higher lung, liver metastasis characteristics which provided a high value on a large animal model studies.

### Experimental methods

#### *The establish of rabbit thigh VX2 transplanted tumor popliteal lymph node metastasis model*

Twelve New Zealand white rabbits were injected with hydrochloric acid chloride acetone solution (50 mg/kg) through left quadriceps and then fixed on the board with supine position after fully anesthesia. The right thigh skin was shaved for preparation, 75% alcohol disinfection and laying sterile towel. Cut the skin to the muscle layer, looking for quadriceps and cut to its deep surface, suture muscle and skin after put 0.5 cm × 0.5 cm rabbit VX2 tumor and gelatin sponge in. The tumor grew rapidly 7–10 days after inoculation and increased to 5 cm on average after 20 days. One month later, enlarged lymph nodes were palpable at the right thigh popliteal fossa of 8 rabbits with diameter of about 1–2 cm with hard texture, poor activity and adhesion to the surrounding skin.

#### *Animal anesthesia*

Eight popliteal fossa enlarged lymph nodes palpable VX2 transplanted tumor rabbits were all injected with hydrochloric acid chloride acetone solution (50 mg/kg) through left quadriceps and then fixed on the board with supine position and with bilateral hind foot back shaving and alcohol disinfection after fully anesthesia.

#### *MRI scan*

GEHDX Signa 3.0 T superconducting MRI scanner was used. Plain scan: the 3D TOF CE-MRA sequence scan, parameters were as follows: Flip Angle 30°, TE 1.6 ms, TR 4.5 ms, field of view (FOV) 280 mm × 280 mm, matrix 360 × 224, thickness 1.0 mm, slash70, NEX 2. Plain scan was made before the injection of contrast agent so as to make contrast with angiography images.

Enhancement scan: 0.4 mL Dextran-DTPA-Gd was injected into the bilateral hind legs first, second and third toes webbed gap respectively. Enhanced MR images were obtained after massage the injection site for 30 seconds with interval 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, 40 min, 45 min, 50 min, 55 min, 60 min, 2 h, 4 h, and 24 h. Scan sequence parameter was associated with plain scan. The rabbits were kept supine position during the entire scanning process. All of the original images were obtained after surrounding background of lymphatic vessels and lymph nodes was eliminated by subtraction technique. Then we got three-dimensional (3D) reconstruction image of rebuilt lymphatic with maximum intensity projection. Signal enhancement ratio (E%) of popliteal fossa lymph nodes was measured and calculated to draw popliteal lymph node signal intensity-time curves. It could be used to observe the development of cancer me-

tastasis lymph nodes and lymphatic and the differences of both side popliteal fossa lymphography.

#### Evaluation

Measured the signal intensity (SI) and system noise (N) of popliteal lymph node with region of interest (ROI) at different sequences MRI image before and after subcutaneous injection. Calculated lymph node SNR and signal intensity ratio (E%) with the formula:  $SNR = SI/N$ ,  $E\% = (SNR_{post} - SNR_{pre}) / SNR_{pre} \times 100\%$ . The selected ROI size should include the entire range of the lymph nodes. SNR<sub>pre</sub> and SNR<sub>post</sub> in the formula represented enhanced scans before and after the popliteal lymph node SNR respectively. Then drew enhancement effect-time curve.

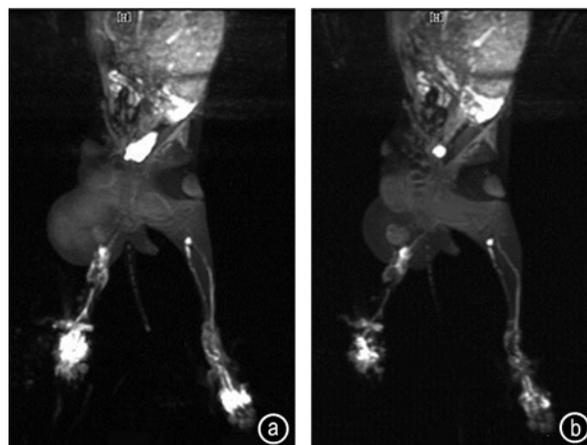
#### Statistical analysis

SPSS 13.0 for Windows statistical software package was used to evaluate the differences of signal intensity over time after enhancement of lymph nodes via repeated measurement data variance analysis. It was considered statistically significant as long as  $P < 0.05$ .

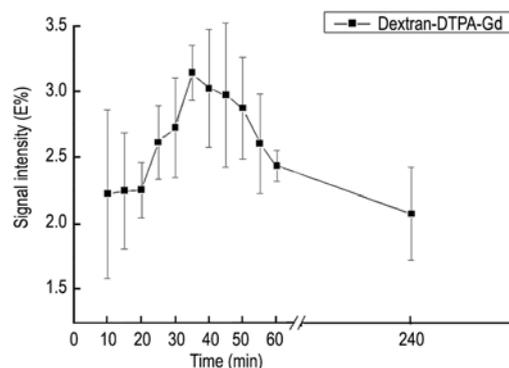
#### Results

The targeted lymphatic contrast agent Dextran-DTPA-Gd was rapidly absorbed into the lymphatic system after injection. Bilateral popliteal lymph nodes and hind legs lymphatic vessels showed clearly after 10 min and the contrast agent gathered at the webbed toe clearance. But there was no blood vessel development. The bilateral popliteal lymph nodes and hind legs lymphatic vessels development was still clear after 20 min with the right side popliteal lymph node rough around like, lymphatic tortuous thickening, lymphatic chain incomplete while the left side popliteal lymph nodes round or oval, clear edge and several lymphatics appeared at hind legs with clear beaded developing. There was still no blood vessel development and the contrast agent gathered at the webbed toe clearance clearly. The development reached a peak at about 35 min (Fig. 1a). After 60 min, the lymph strengthen was still obvious but the lymphatic development diminished and the contrast gent between webbed toes became lighter (Fig. 1b). After 4 h, hind popliteal lymph nodes and lymphatic development weakened and the development between webbed toes was dissection. The others were all eliminated after 24 h.

Results of the signal intensity ratio (E%) and the ratio of enhancement curves of popliteal lymph nodes after subcutaneous injection of Dextran-DTPA-Gd, were shown in Table 1 and Fig. 2.



**Fig. 1** (a) The bilateral popliteal lymph nodes and hind legs lymphatic vessels development was clear after 35 min with the right side popliteal lymph node rough around like, lymphatic tortuous thickening, lymphatic chain incomplete because tumor cell invasion and lymph lymphatic obstruction. (b) After 60 min, the lymph strengthen was still obvious but the lymphatic development diminished.



**Fig. 2** The signal intensity-time curve of popliteal lymph nodes after subcutaneous injection of Dextran-DTPA-Gd

**Table 1** The signal intensity ratio (E%) of popliteal lymph nodes after subcutaneous injection of Dextran-DTPA-Gd

Time	E%
10 min	2.23 ± 0.64
15 min	2.25 ± 0.44
20 min	2.26 ± 0.21
25 min	2.62 ± 0.28
30 min	2.73 ± 0.38
35 min	3.15 ± 0.21
40 min	3.03 ± 0.45
45 min	2.98 ± 0.55
50 min	2.88 ± 0.39
55 min	2.61 ± 0.38
60 min	2.44 ± 0.12
4 h	2.08 ± 0.35
24 h	0

## Discussion

At present, the diagnostic level of lymphatic disease has been greatly improved with imaging examination such as ultrasound, CT, MRI and so on. But there is still no objective standard to evaluate different parts of the lymphatic tissue, lymphatic distribution and out of shape, lymph node morphology as well as small lymph nodes metastasis in tumor patients. Direct lymphography and interstitial lymphoscintigraphy show the precise structures of the lymphatic vessels and lymph nodes due to its partial injury, inflammation, technical difficulties and poor spatial temporal resolution which make it has little clinical application<sup>[9, 10]</sup>. However, IMRLG is a method to inject strongly paramagnetic contrast agent MR into the subcutaneous interstitial which is absorbed by capillary lymphatic drainage area and then remitted to collections lymphatic vessels, lymph nodes, lymph stem and lymphatic ducts via a high-resolution three-dimensional reconstruction of MR scanning to clearly shows the distribution of the lymphatic drainage area, out of shape and lymphatic nodes morphology.

Currently, the enhancement MR contrast agent used at home and abroad clinically are commonly commercialized small molecule gadolinium such as Magnevist, Omniscan which are all strong paramagnetic crystal compound with relative molecular mass less than 1000 Da and diameter of less than 1 nm. In recent years, it has also achieved good results as is used in IMRLG to display lymphatic vessels and lymph nodes of experimental animal limbs. And it has been continually used for the diagnosis of human upper limb lymphedema<sup>[3, 4]</sup>. However, the developing strength of accompanying vein was significantly higher than that of lymphatic due to the small particle size, permeability differences between the wall of lymphatic and capillary, and small molecular crystal less than 10 nm is primarily absorbed and exchange by capillaries, rarely spread through lymphatic<sup>[11]</sup>. While the macromolecules contrast agents commonly used in IMRLG such as albumin-gadopentetate for acid meglumine (HSA-Gd-DTPA), liposomes wrapped gadolinium will take several hours to reach the peak due to its large molecular mass. Although it can be selectively absorbed by lymphatics and lymph nodes, it's not conducive to rapid MR imaging. The lymphatic mainly absorb macromolecules. There are open gaps between the endothelial cells of the capillary lymphatic wall which can be 30–120 nm at stationary state. It can significantly increase with its permeability up to 500 nm while the hydrostatic pressure gradient inside and outside the lymphatic capillary change due to skeletal muscle movement, local injection, massage, or inflammation. The hydraulic differential between lymph inside the capillary lymph tubes and the interstitial fluid out of that is the power to promote lymphatic flow and be ab-

sorbed<sup>[12, 13]</sup>. The size of the contrast agent particle is an important indicator to decide the biological dynamics of lymphatic absorption.

The macromolecular contrast agent Dextran-DTPA-Gd, average molecular size 130 nm, osmotic pressure 77 mOsm/kg·H<sub>2</sub>O and molecular weight 45 000 Da, can quickly enter lymphatic capillaries selectively and make clear popliteal lymph nodes developing due to its molecular diameter is significantly larger than capillary pore<sup>[14]</sup>.

In this experiment, we can see bilateral popliteal lymph nodes and hind legs lymphatic vessels showed clearly after 10 min and the contrast agent gathered at the webbed toe clearance. But there was no blood vessel development. The bilateral popliteal lymph nodes and hind legs lymphatic vessels development was still clear after 20 min with the right side popliteal lymph node rough around like, lymphatic tortuous thickening, lymphatic chain incomplete while the left side popliteal lymph nodes round or oval, clear edge and several lymphatics appeared at hind legs with clear beaded developing. There was still no blood vessel development and the contrast agent gathered at the webbed toe clearance clearly. The development reached a peak at about 35 min. After 60 min, the lymph strengthen was still obvious but the lymphatic development diminished and the contrast agent between webbed toes became lighter.

The experiment proved that, as a contrast agent for IMRLG, Dextran-DTPA-Gd can selectively get into the lymphatic capillary endothelial cell gap, and be rapidly absorbed by the lymphatic duct, reach the peak at about 35 min and maintain a high strengthen rate of nearly 1 h. It can show clear developing of cancer metastasis lymph nodes and lymphatic with the features of irregular shape, ill-defined and inhomogeneous center enhancement which is significantly different compared to normal lymph nodes. It can also display the morphology and out of shape of metastatic lymph nodes quickly and effectively with lymphotropic properties which provides a relevant theoretical basis for the next study of head and neck cancers.

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