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Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2015) xxx–xxx
nanomedicine
Nanotechnology, Biology, and Medicine
nanomedjournal.com

Magnetic sentinel lymph node biopsy in a murine tumour model

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Received 4 October 2015; accepted 10 December 2015

Abstract

The magnetic technique for sentinel node biopsy provides a radioisotope-free alternative for staging breast cancer. This technique requires refinement to reduce the "residual iron content" at the injection site by maximising lymphatic uptake to prevent "void artefacts" on magnetic resonance imaging (MRI), which could adversely affect clinical use. The impact of site and timing of injection of magnetic tracer was evaluated in a murine tumour model (right hind limb) in 24 wild type mice. Right-sided intratumoural and left sided subcutaneous injection of magnetic tracer and assessment of nodal iron uptake on MRI, surgical excision and histopathological grading at time frames up to 24 hours were performed. Rapid iron uptake on MRI, smaller "void artefacts" ($P < 0.001$) and a significant increase in iron content with time were identified in the subcutaneous injection group ($r = 0.937$; $P < 0.001$). A subcutaneous injection and increasing time-delay between tracer injection and surgery is beneficial for lymphatic iron uptake.

Key words: Superparamagnetic iron oxide (SPIO); Magnetic tracer; Magnetic technique; Sentinel lymph node biopsy; Murine tumour model

Introduction

Sentinel lymph node biopsy (SLNB) has been applied as a minimally invasive technique to identify the spread of metastases from the primary tumour to its draining lymph nodes in a range of solid cancers. This technique has been extensively assessed in breast cancer and has become the standard of care — supported by evidence from randomised controlled trials (RCTs) and meta-analyses.[1-6] The current technique, which has provided the highest sentinel node identification, is the "combined technique" of radiolabelled nanocolloid and blue dye.[2] The dependence of this technique upon radioisotopes has resulted in low uptake within institutions lacking nuclear medicine facilities and consequently, non-radioisotope techniques being developed. One of the most promising techniques is the magnetic technique, which uses a magnetic tracer administered interstitially into the breast and identified using a handheld magnetometer. This technique was demonstrated to be non-inferior to the "combined technique" in breast cancer within the phase II, non-randomised, SentiMAG Multicentre Trial[7] and subsequently confirmed in 3 further trials, using the same protocol.[8-10] These trials used 2 mL of magnetic tracer (Sienna+, Endomagnetics Ltd, Cambridge, UK) – diluted with normal saline to 5 mL – injected subcutaneously, periareolarly into the breast. However, magnetic tracers were originally developed as magnetic resonance imaging

Conflicts of interest statement and funding: The authors have no disclosures to make concerning financial and personal relationships with other people or organisations that could inappropriately influence their work. The European Society of Surgical Oncology (ESSO), Young Surgeons and Alumni Club (EYSAC) Research Fellowship (2014-15) provided travel support for MA to conduct this research at the University of Tokyo, Japan.

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http://dx.doi.org/10.1016/j.nano.2015.12.366
1549-9634/

(MRI) contrast agents and therefore are known to create void artefacts on MRI from residual iron content. The extent and length of time such artefacts persist has not been formally evaluated prospectively, but is currently the basis of ongoing research.[11] Due to the "blackish-brown" colour of the magnetic tracer, skin tattooing of patients is also possible. These limitations demonstrated the urgent need to optimise the technique to minimise residual magnetic tracer at the injection site. We previously evaluated the optimisation of this technique within a porcine model with respect to concentration and volume of administration of a magnetic tracer, identifying a significant benefit to a greater concentration (27.5 mg/mL *versus* 11.2 mg/mL) but no similar benefit for greater volumes of magnetic tracer (0.06-2 mL).[12] However, outstanding issues to optimising this technique include the optimal timing of administration before surgery and the location of injection of the magnetic tracer in order to maximise lymphatic uptake and minimising residual iron at the injection site. We therefore assessed these 2 variables within a murine tumour model using correlation between iron quantification, MRI and histopathology.

Methods

Murine tumour model

The University of Tokyo Ethical Review Board granted approval for conducting animal studies (reference number; KA15-2) and all procedures followed were in accordance with institutional guidelines and ensured the humane care of animals. Murine melanoma derived B16-BL6 cells (provided by the Cell Resource Centre for Biomedical Research, Tohoku University, Sendai, Japan) were cultured and maintained in RPMI-1640 containing 10% foetal bovine serum and 1% of antibiotic-antimycotic solution. The B16-BL6 cells (3.0×10^5 cells in 200 μL phosphate buffered saline) were subcutaneously injected into the right hind limb of C57BL/6NCrSlc mice (Japan SLC, Inc., Tokyo, Japan) (24 male, wild type, 11 weeks old) and the tumour model allowed to-develop *in vivo* for 7 days (Figure 1).

The mice were classified into 4 different groups, which underwent injection of a superparamagnetic iron oxide (SPIO) magnetic tracer (Sienna+, Endomagnetics Ltd, Cambridge, UK) at different time intervals before the performance of MRI and surgical excision of inguinal and popliteal lymph nodes, which form the basis of lymphatic drainage of the lower limb. After 7 days from injection of the B16-BL6 cells each mouse was anaesthetised (using a mixture of 2-3% isoflurane and 300 mL/min air via an automatic delivery system (Isolfurane Vaporiser, SN-487 Shinano Manufacturing, Bunkyo-ku, Tokyo, Japan)) and underwent an intratumoural (deep) injection of magnetic tracer (5 μL of magnetic tracer and Patent Blue dye (Guerbet, Paris, France)) in the right hind limb and a subcutaneous (superficial) injection of the same volumes of magnetic tracer and blue dye in the left rear footpad. This was followed by the performance of MRI and surgical lymph node harvesting at 4 different time periods (Group 1: immediately after injection; Group 2: 1 hour after injection; Group 3: 4 hours after injection and Group 4: 24 hours after injection).

Figure 1. Murine tumour model *in vivo* at 7 days in right lower limb.

In vivo visualisation of lymph nodes using MRI

Mice were anaesthetised using a mixture of 2-3% isoflurane and 300 mL/min air via an automatic delivery system (Isolfurane Vaporiser, SN-487 Shinano Manufacturing, Bunkyo-ku, Tokyo, Japan). The mice were repeatedly monitored during the procedure to maintain adequate depth of anaesthesia. The mice were placed in a prone position during imaging. The MRI studies were performed using a Bruker 7.0T BioSpec high-field small animal MRI system with a commercial mouse coil (Bruker Biospin, Ettlingen, Germany). For each animal, T1-weighted (T1W) and T2-weighted (T2W) MRI images were acquired in both coronal and axial orientations. The parameters for these *in vivo* measurements were; T2W: turbo spin echo (TSE) sequence, repetition time (TR)/echo time (TE) = 6844/44 ms; field of view (FOV) = 35×35 mm^2; matrix = 256×256; turbo factor = 8; slice thickness 0.14 mm, isotropic in-plane resolution = 0.14 mm. T1W: segmented turbo-flash gradient-echo (TFL) sequence with an inversion recovery preparatory pulse (IR-TFL), TR/TE = 1300/4.3 ms; FOV =35×35 mm^2; matrix = 256×256; slice thickness = 0.14 mm; isotropic in-plane resolution = 0.14 mm. The T1W and T2W MRI images were acquired without fat suppression. All mice underwent scanning pre and post administration of the magnetic tracer. The visualisation of draining lymph nodes (popliteal and inguinal) and their uptake of SPIO – as seen by the "presence or absence" of hypo-intense changes on MRI – were recorded (Figure 2, *A-B*). The size of the "void artefact" – from residual iron at the injection site – was quantified using 3-dimensional ($l \times h \times w$) measurements from coronal and sagittal sections on MRI (Figure 2, *C-E*).

Lymph node harvesting

Mice were euthanised using cervical dislocation. The mice were fixed in a prone position and inguinal and popliteal lymph nodes – draining the lower limbs – exposed and subsequently surgically excised (Figure 3, *A-B*).