Development of iron oxide nanoparticles for MRI-SPECT-Optical Imaging of sentinel lymph nodes

R. Madru[1], P. Kjellman[2], P. Svenmarker[3], K. Wingårdh[1], S. Fredriksson[2], A. Örbom[1], S. Andersson-Engels[3], C. Ingvar[4], L. Knutsson[1], J. Olsrud[5], J. Lätt[5], F. Ståhlberg[1], and S-E. Strand[1]

[1]Medical Radiation Physics, Lund University, Lund, Sweden, [2]Genovis AB, Lund, Sweden, [3]Physics, Lund University, Lund, Sweden, [4]Surgery, Skane University Hospital, Lund, Sweden, [5]Center for Medical Imaging and Physiology, Skane University Hospital, Lund, Sweden

Purpose

Breast cancer and malignant melanoma spreads through the lymphatic system and metastases can develop in sentinel lymph nodes (SLN), defined as the first node that receives lymph drainage directly from the primary tumor site [1]. The staging and therapy planning of cancer is based on identification and characterization of SLN as a strong prognostic factor. Currently lymphoscintigraphy is the most frequently used method for assessing SLN in cancer staging. The technique relies on subcutaneous injection of 99mTc-nanocolloids followed by static acquisitions of the lymphatic tracer with scintillation camera. A blue dye is injected around the tumor prior to surgery to identify the SLN intra-operatively. The sampled lymph nodes are sent to biopsy at the time of primary tumor excision. This surgical staging is invasive and is limited by surgical field for nodal sampling. To improve this technique we have developed a contrast agent based on superparamagnetic iron oxide nanoparticles (SPIONs) which can be labeled with radioisotopes (for SPECT imaging) or/and fluorophores for optical imaging of the SLN. In this study we present **SLN mapping with MRI/SPECT and/or Optical** and compare the bio distribution of the **^{99m}Tc-SPION** and **^{99m}Tc-SPION-Alexa Fluor 647** agents for translational diagnostic.

Material and methods

The synthesized SPIONs with a solid iron oxide core of 11 nm and coated with polyethylenglycol (PEG) were functionalized with primary amino groups. Alexa Fluor 647 fluorescent dye was conjugated with SPIONs by NHS coupling. An amount of 0.1ml of the agent (3.4mgFe/ml) was then labeled with ^{99m}Tc by stannous reduction of pertechnetate ($^{99m}TcO_4^-$). The incubation time was 1h. Stability and labeling efficiency of the SPIONs in aqueous solution was determined with instant thin layer chromatography (ITLC) using 85% methanol as mobile phase. Subsequently a second solution with **^{99m}Tc-ION** using the protocol described above without the fluorophore was prepared in order to compare the uptake and distribution of the contrast agents in vivo. Five Wistar rats were injected subcutaneously (s.c.) in the right back pad with 0.07-0.1ml (40-65MBq) **^{99m}Tc-ION** while one rat was injected with 0.1ml (65MBq) **^{99m}Tc-ION-Alexa Fluor 647** in aqueous solution. Four hour post injection the animals were sacrificed and imaged with MRI, SPECT and Optical system. The MR images were obtained with a 3T MR unit supplied with a Sense-Flex-M coil. Spin echo and gradient echo sequences were used for a detailed anatomical visualization of the SLN respective uptake of the contrast agent. In order to achieve high signal-to-noise ratio (SNR) we also used a balanced fast field echo sequence, successfully applied for diagnostic- and functional imaging in the clinic. This sequence provides a T2*/T1 tissue weighting often used for blood to tissue contrast. The imaging parameters were as follows: SE, TE/TR=80/3000ms, FOV 100mm, slice thickness 1.3mm, slice gap 1.3mm, flip angle 90°, NSA 10; GRE T1w, TE/TR=4/9ms, FOV 100mm, slice thickness 1.3mm, slice gap 1.3mm, flip angle 10°, NSA 10; GRE T2w, TE/TR=15/2000ms, FOV 78mm, slice thickness 1.3mm, slice gap 1.3mm, flip angle 90°, NSA 10. In order to quantify the contrast agent uptake and to study the bio distribution each animal was dissected and organs as lymph nodes, bladder, kidneys, spleen, liver and the injection site were removed, weighted and measured for activity.

Results

Both **^{99m}Tc-SPION** and **^{99m}Tc-SPION-Alexa Fluor 647 contrast agents** show a colloidal stability of more than 6h determined with ITLC. The labeling efficiency of the SPIONs with ^{99m}Tc was found as high as 99%. The SLN could be detected using all three modalities however the quantification of the amount of SPIONs leaving the injection site and bio distribution could not be done without the radioisotope.

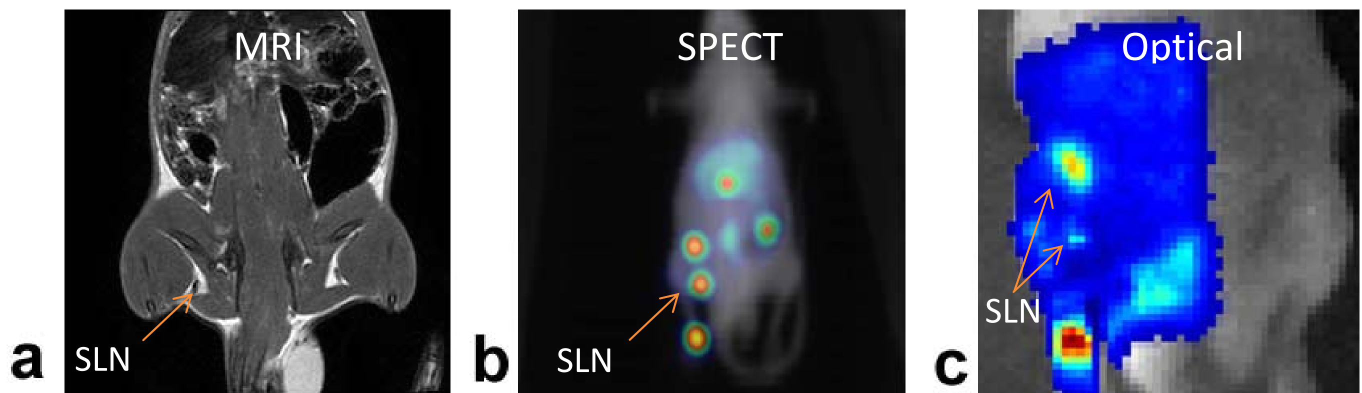

Figure 1. Visualization of the SLN using ^{99m}Tc-SPION-Alexa Fluor 647 contrast agent with (a) MRI, spin echo image, (b) SPECT and (c) Optical image system

The distribution of ^{99m}Tc-SPION and ^{99m}Tc-SPION-Alexa Fluor 647 contrast agents in vivo was similar. Four hours after the administration of the SPIONs about 7% leaved the injection site whereas 3.5% of the injected activity was found in SLN. Accumulation of the SPIONs was also detected in the lumbar (0.7%), inguinal (0.1%) and mediastinal (0.15%) lymph nodes, spleen (0.2%), kidneys (0.4%), urine (0.2%) and liver (1.4%).

Conclusion

We have developed a multimodality imaging contrast agent based on iron oxide nanoparticles for SLN mapping. We have shown that the contrast agent has a good colloidal stability and great potential for in vivo imaging. The different modalities complement each other well. The high resolution and detailed anatomical MR images will facilitate the treatment planning and detection the SLN. The ^{99m}Tc is a well-established radioisotope in clinical application and can be used for obtaining high sensitivity SPECT images that we in the future plan to co-registrate with MR images. The fluorescent tag will be used for intra-operative or bed-side detection of the nodes, helping the surgeon to find SLN during the operation.

Reference [1] Dunitz M. Sentinel lymph node biopsy. 2002. ISBN 1841840343