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SHORT COMMUNICATION

First ex vivo study demonstrating that ^{99m}Tc-NTP 15-5 radiotracer binds to human articular cartilage

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Abstract
Purpose Preclinical data pointed to ^{99m}Tc-NTP 15-5 as a good candidate for single photon emission computed tomography (SPECT) imaging of cartilaginous disease. We set out to investigate and quantify ^{99m}Tc-NTP 15-5 ex vivo uptake by human articular cartilage relative to bone ^{99m}Tc-hydroxymethylene diphosphonate (HMDP) radiotracer.
Methods Three osteoarthritic human tibial plateaux and four tibiofemoral joints were incubated with ^{99m}Tc-NTP 15-5 and ^{99m}Tc-HMDP for 2 h. Affinity of tracers for cartilage was determined by visual analysis of SPECT/CT acquisitions and measurement of cartilage to cortical bone uptake ratios.
Results Cartilage to cortical bone uptake ratios were 3.90±2.35 and 0.76±0.24, respectively, for ^{99m}Tc-NTP 15-5 and ^{99m}Tc-HMDP radiotracers. Visual analysis of fused SPECT/CT slices showed selective, intense ^{99m}Tc-NTP 15-5 accumulation in articular cartilage, whereas ^{99m}Tc-HMDP binding was low. Interestingly, a cartilage defect visualized on CT was clearly associated with focal decreased uptake of ^{99m}Tc-NTP 15-5.
Conclusion The tracer ^{99m}Tc-NTP 15-5 is of major interest for human cartilage molecular imaging and could find clinical applications in osteoarthritis staging and monitoring.

Keywords Proteoglycans · Osteoarthritis · SPECT/CT · ^{99m}Tc-NTP 15-5 radiotracer

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Introduction

Our group has developed ^{99m}Tc-*N*-(triethylammonium)-3-propyl-[15]ane-N5 (^{99m}Tc-NTP 15-5), which binds to proteoglycans (PG) in cartilage [1]. Potential clinical applications include the assessment of degenerative, inflammatory and malignant cartilage diseases.

Many studies have already been conducted for the characterization and preclinical validation of ^{99m}Tc-NTP 15-5. A high selective tracer accumulation in normal articular cartilage was confirmed in several animal species [2, 3]. In experimental osteoarthropathy models, ^{99m}Tc-NTP 15-5 was evaluated relative to ^{99m}Tc-hydroxymethylene diphosphonate (HMDP) imaging. ^{99m}Tc-NTP 15-5 uptake was correlated with age and joint disease severity [4], while ^{99m}Tc-HMDP imaging evidenced bone remodelling

in advanced stages. On a meniscectomized guinea pig model, an initially increased ^{99m}Tc-NTP 15-5 uptake was observed in the pathological joint, followed by a decreased tracer uptake at the late stage [3]. The initially high tracer accumulation corresponded to PG newly synthesized in response to the articular aggression [5]. In the orthotopic Swarm rat chondrosarcoma model, ^{99m}Tc-NTP 15-5 imaging evidenced a high uptake of the tracer at the tumour site [6].

These preclinical results showed ^{99m}Tc-NTP 15-5 to be a good candidate single photon emission computed tomography (SPECT) tracer for the functional imaging of cartilage in nuclear medicine. Before clinical transfer to humans, we evaluated the potential of the radiotracer to bind ex vivo to human articular cartilage. Osteoarthritic knee parts provided from prosthetic surgery and large knee specimens obtained from the anatomy laboratory were incubated with ^{99m}Tc-NTP 15-5 and ^{99m}Tc-HMDP, respectively. SPECT and CT imaging were performed with hybrid or separate facilities to evaluate (1) tracer accumulation in target and non-target tissues, (2) localization of radioactive signal in anatomical structures and (3) quantification of cartilage to bone uptake ratios.

Materials and methods

Radiolabelled tracers

^{99m}Tc-NTP 15-5 was prepared and radiolabelled as previously described [4], with a specific radioactivity of 25 MBq.μmol^{-1}. ^{99m}Tc-HMDP was prepared using the Osteocyt® kit (IBA, CIS Bio International, Gif-sur-Yvette, France).

Ex vivo uptake studies

Tibial plateaux (three specimens) removed during prosthetic surgery (Fig. 1) and inferior femoral joints (four large specimens) from the anatomy laboratory were kept in a solution of phosphate-buffered saline (PBS) and ethanol at +4°C. For incubation, specimens were immersed for 2 h in 0.9% saline solution containing 74 or 148 MBq of ^{99m}Tc-NTP 15-5 or ^{99m}Tc-HMDP, respectively. Specimens were then washed for 2 h with 0.9% physiological saline solution and dried before acquisition. For each sample, the residual activity was taken up from both the incubation medium and washing solutions for counting by planar imaging (see below).

Planar and SPECT/CT imaging

The incubated dose was determined from planar acquisition (2 min duration) of incubated medium and washing solutions.

Three-dimensional SPECT/CT images of incubated specimens were acquired to visualize and quantify the radiotracer uptakes within cartilage and bone.

The gamma camera used for acquisition depended on the size of the specimen:

- For *small prosthetic specimens*, acquisitions were performed with a *dedicated small animal camera* (γ Imager, Biospace Lab, Paris, France). SPECT acquisition (45 min duration, pixel size 390 μm) was performed with articular specimens and two linear fiducial markers placed on a dedicated rotating platform. Tomographic reconstruction was performed using a validated ordered subset expectation maximization (OSEM) algorithm. Immediately after SPECT acquisitions, 0.625-mm thick CT slices (GE Discovery ST, GE Healthcare, 120 kV, 600 mAs, pixel size 0.195 mm) were acquired. Fusion and quantification were performed using AMIDE software [7].
- For *large specimens*, SPECT/CT imaging was performed using a clinical hybrid facility (Symbia T, Siemens). For SPECT, 64 projections were acquired, with the duration of each projection being 40 s. An OSEM algorithm was used for slice reconstruction. For CT, 1-mm thick CT slices (80 kV, 45 mAs, pixel size 0.273 mm) were acquired and automatically fused with SPECT slices.

Image analysis

Images were analysed for both the visual identification of anatomical structures evidencing tracer uptake and quantification of radioactivity. The volume (cm^3) of the whole specimen was determined from CT using a region growing algorithm. Cartilage, cortical and medullary bone regions of interest (ROIs) were drawn manually on CT. These regions were duplicated on the corresponding SPECT slices and the mean activity of each ROI was quantified.

For each radiotracer, results were expressed as percentage of incubated dose (%ID), cartilage to cortical (cart/cort) and cartilage to medullary (cart/med) bone uptake ratios. ^{99m}Tc-HMDP and ^{99m}Tc-NTP 15-5 uptake ratios were compared each other with the Mann-Whitney test. Due to the small number of specimens, no comparison was performed in the subgroups of small or large specimens ($n<5$).

Results

Percentage of incubated dose

The mean %ID was 3.59±1.11% and 7.81±4.34%, respectively, for ^{99m}Tc-NTP 15-5 and ^{99m}Tc-HMDP. Volume and %ID per specimen are summarized in Table 1.

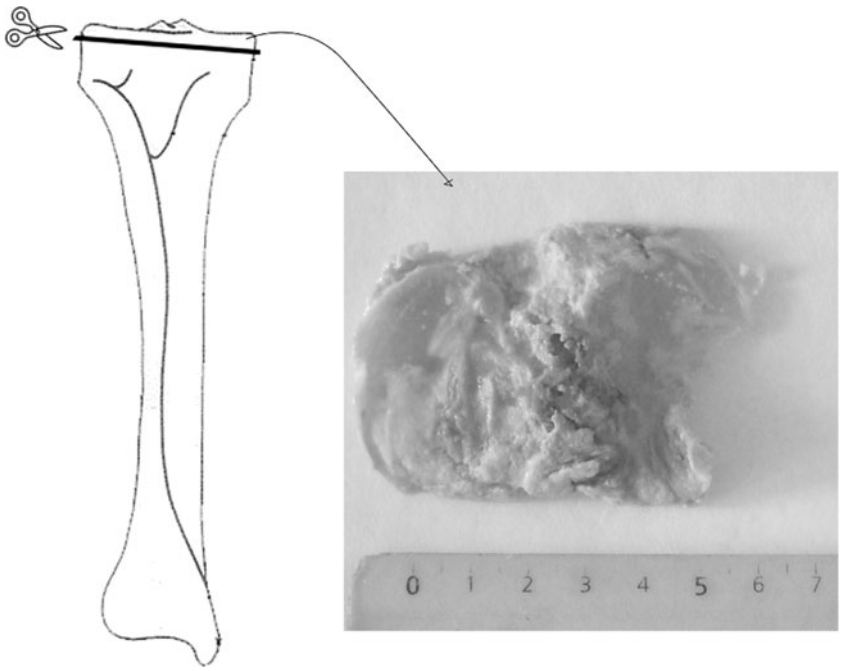

Fig. 1 Specimens obtained during a total knee replacement procedure. The surgeon cut the upper part of the tibia (*left*) to remove the osteoarthritis plateau (*right*). This excised plateau was used for the imaging study. Note the absence of cartilage on the left part of the plateau

Cart/bone uptake ratios

- Considering the large specimen population, mean cart/cort and cart/med uptake ratios observed after ^{99m}Tc-NTP 15-5 incubation were 4.57±3.07 and 23.21±2.6, respectively. With ^{99m}Tc-HMDP incubation, mean ratios were 0.71±0.31 and 7.14±4.88, respectively.
- For small specimens, mean cart/cort and cart/med uptake ratios calculated after ^{99m}Tc-NTP 15-5 incubation were 3.01±0.59 and 3.07±0.42, respectively. After ^{99m}Tc-HMDP incubation, mean ratios were 0.82±0.11and 1.66±0.79, respectively.
- Considering the whole population of specimens, mean cart/cort uptake ratio determined from ^{99m}Tc-NTP 15-5 scintigrams was significantly higher than the ratio

Table 1 Radiotracer uptake expressed as %ID, cartilage to bone and cartilage to medullary uptake ratios after ^{99m}Tc-NTP 15-5 (NTP) and ^{99m}Tc-HMDP (HMDP) incubation of human articular specimens. Uptake ratios were calculated for each specimen, averaged per specimen size category (large and small) and pooled for the overall population

Tracer		Specimen	Volume (cm^3)	%ID	Cart/cort			Cart/med		
					Per specimen	Per group (mean ± SD)	Overall population	Per specimen	Per group (mean ± SD)	Overall population
NTP	SS group	Tibia	28.34	5.56	2.53	3.01±0.59	3.90±2.35	2.80	3.07±0.42	14.58±10.92
		Tibia	11.06	3.84	2.83			2.86		
		Tibia	10.35	2.77	3.66			3.55		
	LS group	Tibia	156.57	2.01	1.78	4.57±3.07		26.43	23.21±2.6	
		Femur	216.31	3.65	2.11			22.98		
		Tibia	166.10	4.02	7.80			23.34		
		Femur	222.86	3.29	6.59			20.08		
HMDP	SS group	Tibia	28.16	6.14	0.90	0.82±0.11	0.76±0.24	2.54	1.66±0.79	4.79±4.55
		Tibia	11.65	9.39	0.86			1.44		
		Tibia	10.60	16.76	0.69			1.00		
	LS group	Tibia	155.62	6.13	1.13	0.71±0.31		13.54	7.14±4.88	
		Femur	215.90	3.18	0.77			8.36		
		Tibia	164.30	6.80	0.42			3.07		
		Femur	219.36	6.24	0.53			3.60		

%ID percentage of the incubated dose, *Cart* articular cartilage, *cort* cortical bone, *med* medullary bone, *SS* small specimen, *LS* large specimen

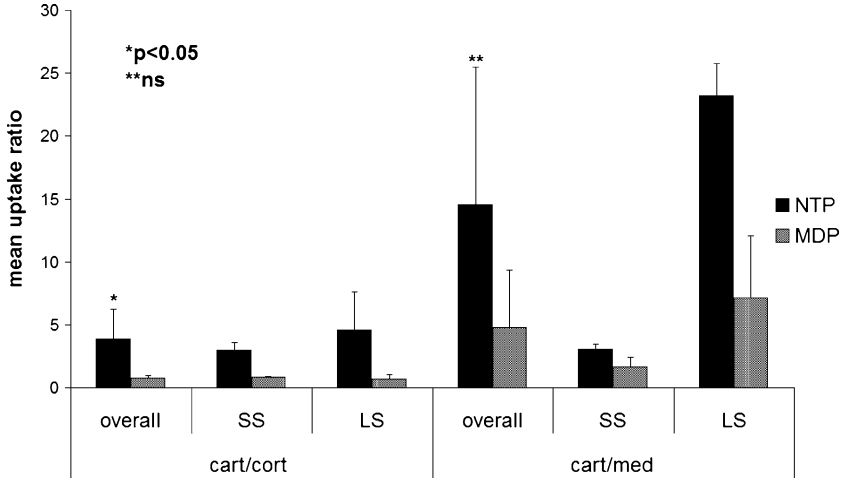

Fig. 2 Mean cartilage/bone (*cart/cort*) and cartilage/medullary (*cart/med*) uptake ratios measured on three small human tibial plateau specimens (SS) and four large femorotibial specimens (LS) incubated with ^{99m}Tc-NTP 15-5 and ^{99m}Tc-HMDP, respectively. Statistical comparison was performed only for the overall population ($n>5$)

calculated from ^{99m}Tc-HMDP images (3.90±2.35 vs 0.76±0.24, respectively, $p<0.01$). Mean cart/med uptake ratio tended to be higher with ^{99m}Tc-NTP 15-5 than with ^{99m}Tc-HMDP but failed to reach statistical significance (14.58±10.92 and 4.79±4.55, $p=$ NS). Mean uptake ratios are summarized in Table 1 and Fig. 2.

Visual analysis

Due to the high contrast between air and tissues, cartilage and bone structures were easily identified on CT scans in all of the specimens analysed. From the outer to the inner parts of samples, slice analysis showed three different structures corresponding to (1) cartilage, (2) a thin layer of subchondral bone with high HU density and (3) medullary bone with low HU density (Figs. 3–5). In the diaphysis part of the large specimens, only cortical and medullary bone were visualized.

When the small specimens were incubated with ^{99m}Tc-NTP 15-5, SPECT/CT images evidenced a selective, intense tracer uptake in cartilage (Fig. 3). For larger specimens, ^{99m}Tc-NTP 15-5 images showed a typical accumulation shape delineating the femoral condyle, the tibial plateau and the patellar surface (Fig. 4b, online resource 1). ^{99m}Tc-NTP 15-5 uptake was not marked in surrounding muscle soft tissue, subchondral bone, cortical bone or knee ligament insertion. ^{99m}Tc-NTP 15-5 defects were depicted in the cartilage surface of the medial femoral condyle and were clearly colocalized with the anatomical area of altered cartilage (Fig. 5).

Interestingly, totally different patterns were obtained when samples were incubated with ^{99m}Tc-HMDP. For large specimens, diffuse ^{99m}Tc-HMDP uptake was observed along the bone surface: the highest intensity was localized on the cortical surface, while the lowest accumulation was observed in cartilage. For small specimens, the ^{99m}Tc-HMDP signal was heterogeneously distributed, with no specificity for cartilage or bone tissue (Fig. 4a).

Discussion

Osteoarthritis (OA) in the ageing Western population is a major health problem [8]. The limited treatment available (analgesics, nonsteroidal anti-inflammatory drugs) emphasizes the urgent need to elucidate the molecular mechanisms of disease progression. Disease-modifying osteoarthritis drugs (DMOADs) [9], which can interfere with joint remodelling and destruction, offer new promising therapeutic strategies. However, objective evaluation of treatment efficacy is hampered by the lack of diagnostic tools for assessing early stages of the disease, especially when its progression is to be controlled [8, 10]. Imaging with a labelled specific ligand allowing quantification of cartilage proteoglycan in vivo would be useful for the early detection

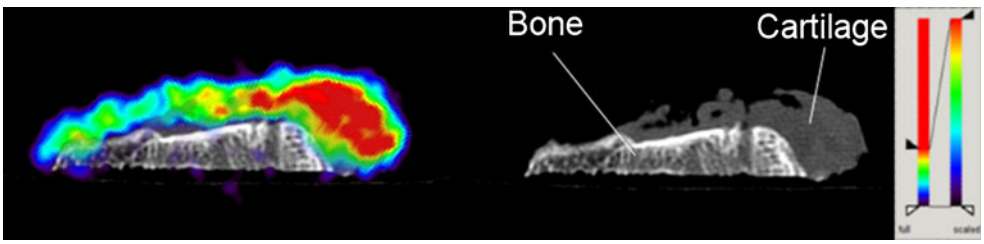

Fig. 3 ^{99m}Tc-NTP 15-5 SPECT/CT fused axial slices (*left*) and CT slices (*right*) of a human tibial plateau specimen. The radioactive signal was mainly colocalized with cartilage tissue

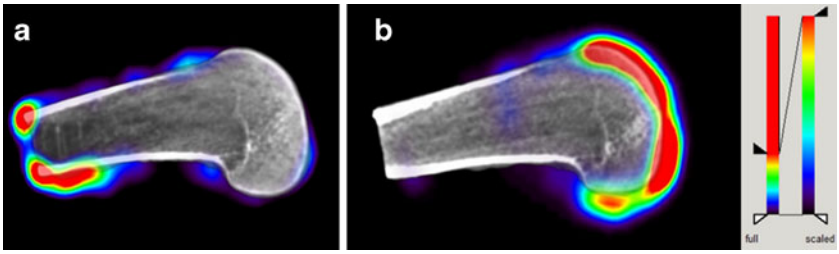

Fig. 4 ^{99m}Tc-HMDP (**a**) and ^{99m}Tc-NTP 15-5 (**b**) SPECT/CT fused sagittal slices of a human femoral condyle specimen. A high accumulation of ^{99m}Tc-NTP 15-5 was visualized over the cartilage surface of the femoral condyle, while only a faint accumulation of ^{99m}Tc-NTP 15-5 was noted on the surrounding soft tissues and cortical bone. When specimens were incubated with ^{99m}Tc-HMDP, the radioactive signal was depicted on the cortical surface of the diaphysis

of arthrosis, longitudinal follow-up of treated patients and evaluation of new therapies.

Many preclinical results showed ^{99m}Tc-NTP 15-5 to be a good candidate for SPECT imaging of cartilage. Bridging

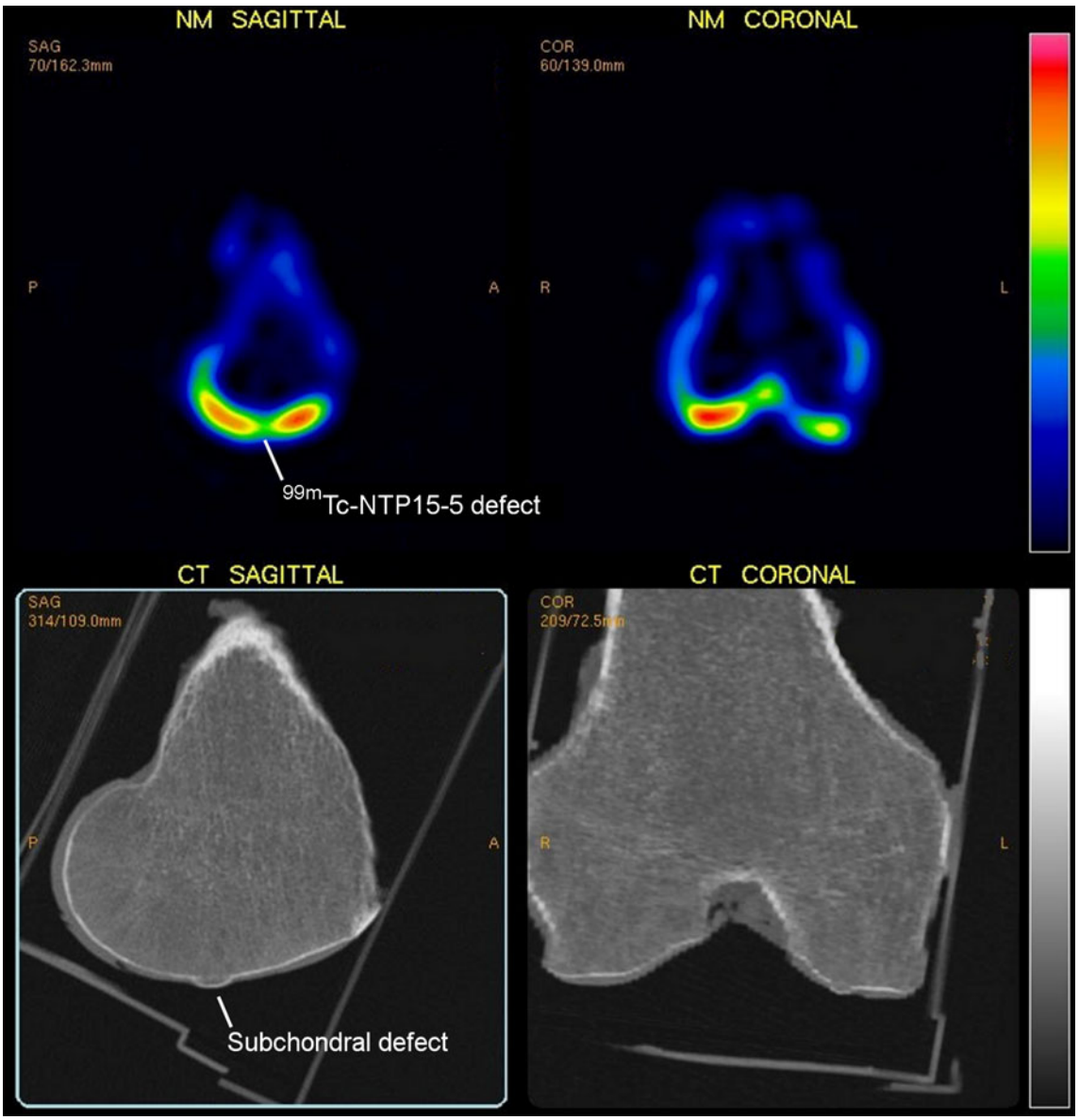

Fig. 5 ^{99m}Tc-NTP 15-5 sagittal and coronal SPECT/CT fused slices of a human femoral condyle. Note that a focal decreased uptake of ^{99m}Tc-NTP 15-5 corresponded to a 7-mm subchondral defect (grade 4) on the CT slice

preclinical and clinical studies, this ex vivo study set out to characterize ^{99m}Tc-NTP 15-5 uptake by human articular specimens. Considering the whole population of specimens, higher tracer accumulation was observed in cartilage than in cortical bone (mean cart/cort=3.90±2.35). By contrast, specimens incubated with the bone radiotracer evidenced only weak ^{99m}Tc-HMDP accumulation (mean cart/cort=0.76 ±0.24). Preferential ^{99m}Tc-NTP 15-5 accumulation in the cartilage was easily demonstrated with large specimens (mean cart/med ratio=23.21±2.6). The difficulty in delineating the medullary part of the bone on the small specimens (2 cm thick) probably explains the relatively low cart/med uptake ratio (mean value=3.07±0.42). Although a passive surface deposit cannot be excluded, ratio values demonstrate that ^{99m}Tc-NTP 15-5 and ^{99m}Tc-HMDP accumulate preferentially in the cartilage and cortical bone, respectively. Analysis of SPECT/CT images confirmed selective, intense ^{99m}Tc-NTP 15-5 accumulation in cartilage. Interestingly, tracer defect areas could be clearly colocalized on CT with anatomical areas of altered cartilage. Although ex vivo tissue incubation with binding tracer cannot be considered as a model for in vivo targeting, this ex vivo study provides additional data to support the "proof of concept".

These experimental results argue in favour of ^{99m}Tc-NTP 15-5 as the first and only radiopharmaceutical available for functional direct imaging of OA at the proteoglycan level. To date, radiotracers currently available for clinical OA imaging provide indirect evaluation of the pathology, such as bone remodelling and inflammation, which can be useful for therapy orientation [10]. However, they are not early indicators of OA and are not specific enough to evaluate disease progression. Our results suggest that ^{99m}Tc-NTP 15-5 is a good candidate functional imaging tracer for OA. The high quality of ^{99m}Tc-NTP 15-5 SPECT/CT images prompt us to initiate clinical transfer.

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Conflicts of interest None.

References

1. Maurizis JC, Rapp M, Nicolas C, Ollier M, Verny M, Madelmont JC. Disposition in rats of N-pyridinium-propyl-cyclam, N-triethylammonium-propyl-cyclam, and N-[triethylammonium]-3-propyl-[15]ane-N5, potential cartilage imaging agents. Drug Metab Dispos 2000;28:418–22.
2. Miot-Noirault E, Vidal A, Auzeloux P, Madelmont J, Maublant J, Moins N. First in vivo SPECT imaging of mouse femorotibial cartilage using 99mTc-NTP 15-5. Mol Imaging 2008;7:263–71.
3. Miot-Noirault E, Vidal A, Pastoureau P, Bonafous J, Chomel A, Sarry L, et al. Early detection and monitoring of cartilage alteration in the experimental meniscectomised guinea pig model of osteoarthritis by 99mTc-NTP 15-5 scintigraphy. Eur J Nucl Med Mol Imaging 2007;34:1280–90.
4. Ollier M, Maurizis JC, Nicolas C, Bonafous J, de Latour M, Veyre A, et al. Joint scintigraphy in rabbits with 99mTc-N-[3-(triethylammonio)propyl]-15ane-N5, a new radiodiagnostic agent for articular cartilage imaging. J Nucl Med 2001;42:141–5.
5. Borel M, Pastoureau P, Papon J, Madelmont JC, Moins N, Maublant J, et al. Longitudinal profiling of articular cartilage degradation in osteoarthritis by high-resolution magic angle spinning 1H NMR spectroscopy: experimental study in the meniscectomized guinea pig model. J Proteome Res 2009;8:2594–600.
6. Miot-Noirault E, Gouin F, Vidal A, Rapp M, Maublant J, Askienazy S, et al. First preclinical imaging of primary cartilage neoplasm and its local recurrence using 99mTc-NTP 15-5 radiotracer. J Nucl Med 2009;50:1541–7.
7. Loening AM, Gambhir SS. AMIDE: a free software tool for multimodality medical image analysis. Mol Imaging 2003;2:131–7.
8. Pelletier JP, Martel-Pelletier J. Therapeutic targets in osteoarthritis: from today to tomorrow with new imaging technologies. Ann Rheum Dis 2003;62(Suppl 2):ii79–82.
9. Qvist P, Bay-Jensen AC, Christiansen C, Dam EB, Pastoureau P, Karsdal MA. The disease modifying osteoarthritis drug (DMOAD): is it in the horizon? Pharmacol Res 2008;58 (1):1–7.
10. Mazzuca SA, Brandt KD, Schauwecker DS, Buckwalter KA, Katz BP, Meyer JM, et al. Bone scintigraphy is not a better predictor of progression of knee osteoarthritis than Kellgren and Lawrence grade. J Rheumatol 2004;31:329–32.