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Pre vivo, ex vivo and in vivo evaluations of [^{68}Ga]-EDTMP

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Abstract

Introduction: The objectives of this study were to develop a simple preparation method for [^{68}Ga]-EDTMP and to evaluate the applicability of [^{68}Ga]-EDTMP as a potential positron emission tomography (PET) bone imaging agent using pre vivo, ex vivo and in vivo models.
Methods: [^{68}Ga]-EDTMP was prepared using [^{68}Ga]-gallium chloride eluted from the ^{68}Ge/^{68}Ga generator and commercially available Multibone kits. Binding affinity to bone compartments was evaluated using a recently established pre vivo model. In vivo (microPET) and ex vivo experiments were performed in mice, and the results of which were compared with those obtained with [^{18}F]-fluoride.
Results: [^{68}Ga]-EDTMP was accessible via simple kit preparation and predominantly accumulated in bone tissue in vivo, ex vivo and pre vivo. Binding to mineral bone was irreversible, and low binding was observed in organic bone. In vivo microPET evaluation revealed predominant uptake in bone with renal excretion. Compared with [^{18}F]-fluoride, the uptake was lower and the PET image quality was reduced.
Conclusions: From the present evaluation, apart from the autonomy for PET centers without an onsite cyclotron, the advantage of [^{68}Ga]-EDTMP over [^{18}F]-fluoride is not apparent and the future clinical prospect of [^{68}Ga]-EDTMP remains speculative.

Keywords: Bone; PET; Gallium-68; Fluorine-18; Bone scan

1. Introduction

Several primary tumors, such as those of the breast, lung and prostate, are known to metastasize into osseous tissue; the detection of these bone metastases plays an important role in medical imaging techniques. One detection method can be bone scanning using polyphosphonates (PPs) mainly radioactivity labeled with technetium-99m. These PPs have already been described in the early 1970s [1], and a variety of [^{99m}Tc]-PPs have been introduced into nuclear medicine thus far (e.g., MDP, DPD, HDP, and EDTMP). Besides these ligand-based bone seekers, [^{18}F]-fluoride, first described in 1962 [2], enjoys its renaissance. Although [^{18}F]-fluoride was introduced more than 10 years ahead of [^{99m}Tc]-PPs, it was replaced by the classical bone imaging tracers for nearly two decades. As compared with [^{99m}Tc]-PPs, [^{18}F]-fluoride has the potential advantages of higher sensitivity and — due to the advanced positron emission tomography (PET) technology — higher spatial resolution [3]. A potential problem could lie in its very high sensitivity in that [^{18}F]-fluoride could give false-positive findings in minimal degenerative changes [4]. Additionally, it had been discussed that [^{18}F]-fluoride uptake represents blood flow rather than bone remodeling [5]. A combination of the advantages of both aspects — ligand-based tracers and

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radionuclides enabling PET — could yield improved diagnostic accuracy.

Another candidate positron emitter, being suggested for PET bone scanning, is gallium-68 [5]. Gallium-68 has been introduced as a generator-produced radionuclide [6], and [^{68}Ga]-EDTMP was described in 1976 [7] and 1978 [8]. Unfortunately, due to several inconveniences with the first generation of ^{68}Ge/^{68}Ga generators, [^{68}Ga]-EDTMP did not find its way into clinical use. However, recent advances in generator design, together with the commercial availability of ^{68}Ge/^{68}Ga generators, increase interest in gallium-68-radiolabeled PET tracers. Hence, [^{68}Ga]-EDTMP could be a valuable alternative to [^{18}F]-fluoride for PET centers without an onsite cyclotron.

The aims of the present study were as follows:

1. simple routine kit preparation of [^{68}Ga]-EDTMP;
2. evaluation of [^{68}Ga]-EDTMP using our pre vivo model [9–11];
3. in vivo comparison of [^{68}Ga]-EDTMP and [^{18}F]-fluoride using microPET in mice; and
4. ex vivo evaluation of the biodistribution of [^{68}Ga]-EDTMP as compared with that of [^{18}F]-fluoride in mice.

Table 1
Binding of [^{68}Ga]-EDTMP and [^{68}Ga]-gallium chloride on filter, 3 mg of HA, Co and D-Co after 120 min

Radiotracer	Percentage of binding on matrix (mean±S.D.)			
	Filter value	HA	Co	D-Co
[^{68}Ga]-EDTMP	5.6±1.5	4.29±2.74	5.44±1.99	1.08±1.89
[^{68}Ga]-gallium chloride	4.9±1.5	59.2±7.1	68.6±8.0	40.3±7.8

Each value represents the arithmetic mean of five experiments, with each measurement performed in triplicate.

2. Materials and methods

2.1. Materials

Multibone kits (containing 25 mg of EDTMP in lyophilized form) were commercially obtained (Izotop, Budapest, Hungary). Hank's balanced salt solution (HBSS, H 8264) and hydroxyapatite (HA, 21223) were purchased from Sigma-Aldrich (Steinheim, Germany). Millex-GS 0.22-μm sterile filters were obtained from Millipore (Bedford, MA, USA). Measurements of radioactivity were performed on a Cobra-II auto-gamma counter (Canberra Packard, Mississauga, ON, Canada). The thermostatic water bath was from GFL (GFL 1083; Burgwedel, Germany), and the dose calibrator was from PTW (Curiementor 2; Freiburg, Germany). The 1110-MBq ^{68}Ge/^{68}Ga generator was obtained from IDB Holland (Baarle-Nassau, The Netherlands). Instant thin-layer chromatography (ITLC)/silica gel (SG) strips were from Gelman (Ann Arbor, MI, USA); autoradiography was performed on an Instant Imager (Canberra Packard).

2.2. Radiotracer preparation

The ^{68}Ge/^{68}Ga generator was eluted with 5 ml of 0.1-N hydrochloric acid (330–520 MBq). Afterward, 2 ml of this eluate was diluted with 3 ml of 0.9% sodium chloride solution, added to a Multibone kit and kept at an ambient temperature for 30 min. Finally, the pH level was adjusted with 1 M of sodium acetate to 5.5–6 (~0.5 ml) and the product was sterile filtrated. Radiolabeling was assessed with ascending ITLC/SG 1×8-cm strips (methanol/ammonium acetate 1 M; 1+1) for each step of the experiments.

2.3. Binding experiments (pre vivo model)

Binding experiments on HA, human cortical bone allografts (Co) and decalcified human cortical bone allografts (D-Co) were performed as described [9–11]. Briefly, 3 mg of their respective powdered matrix was incubated with 0.3 μmol of the ligand-based radiotracers ([^{68}Ga]-EDTMP, [^{90}Y]-EDTMP, [^{99m}Tc]-EDTMP, [^{111}In]-EDTMP, [^{153}Sm]-EDTMP and [^{188}Re]-EDTMP) or 25 MBq of [^{18}F]-fluoride or [^{68}Ga]-gallium chloride in 3 ml of HBSS and then kept at 37°C for 120 min. Percentage of binding was assessed by filtering and subsequent gamma counting [9–12]. Binding values were iteratively corrected for radioactivity remaining in the filter.

2.4. MicroPET experiments

All experiments were approved by the Austrian law on animal experiments. All data were acquired on a microPET Focus 220 tomograph (Siemens, Knoxville, TN, USA) [13] using 6-week-old female wild-type Him:OF1 mice. The PET imaging field of view (FOV) was 190 mm in diameter in the transverse by 76 mm in length in the axial direction. A butterfly catheter was placed in the tail vein, and each mouse was positioned on the thermostatized animal bed (37°C) in the microPET scanner and then kept under isoflurane anesthesia (1.57%). After radiotracer administration (0.2 ml, 15 s) of 0.31–2.05 MBq of [^{68}Ga]-EDTMP or 1.01–4.17 MBq of [^{18}F]-fluoride, data acquisition was started. The dynamic image data (energy window=250–750 keV, timing window=6 ns) were sorted into three-dimensional sinograms using a span of three and a ring difference of 47 (frames=7×1, 4×2, 3×5, 3×10 and 8×15 min).

All sinograms were Fourier transformed into two-dimensional sinograms prior to reconstruction. Dynamic images were reconstructed using two-dimensional filtered back projection with a ramp filter cutoff at the Nyquist frequency. Transmission scans using a Co-57 point source were performed for 10 min. Emission data were corrected for detector efficiency, random coincidences, dead time, isotope decay and attenuation. The PET image volume (128×128×95) was reconstructed with a zoom of 6 and had a voxel size of 0.32×0.32×0.8 mm^3.

2.5. Quantitative image analysis

Calibration factors for converting arbitrary units on microPET images into absolute tracer concentrations were

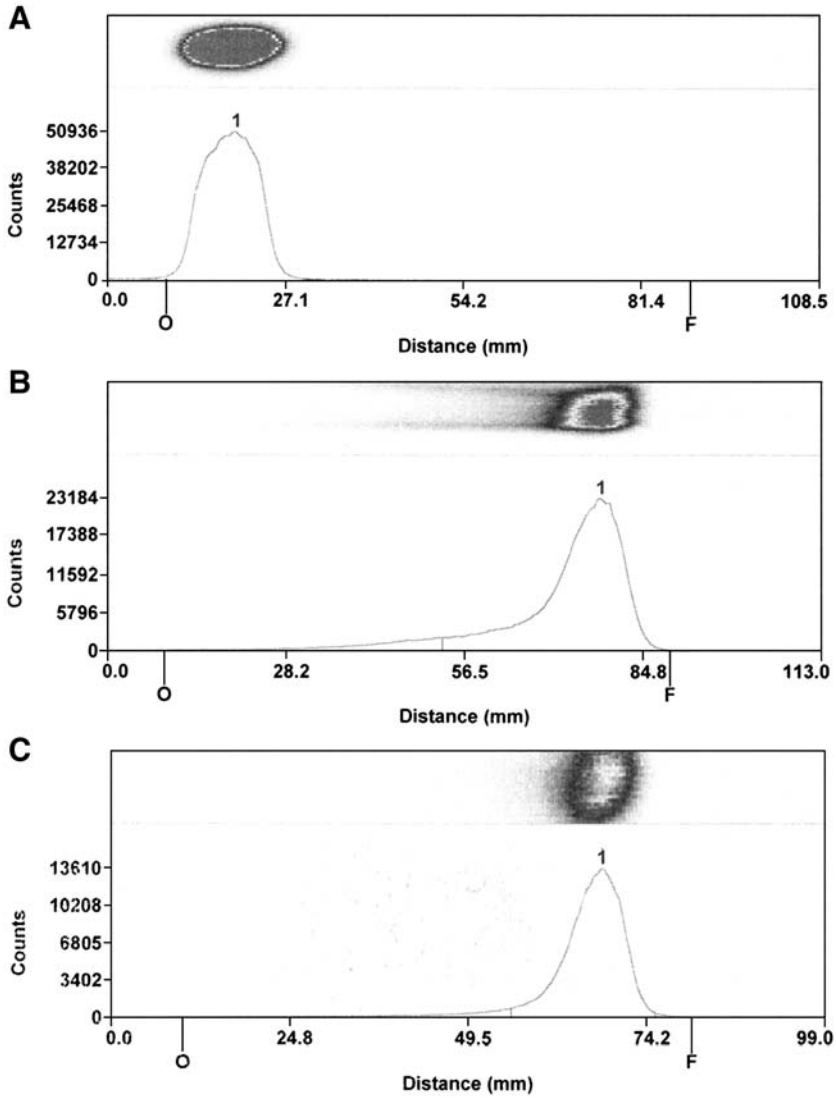

Fig. 1. Illustrative example of the quality control of [^{68}Ga]-EDTMP. (A) Free [^{68}Ga]-gallium. (B) [^{68}Ga]-EDTMP after 5 min. (C) [^{68}Ga]-EDTMP after 300 min (O = start, F = solvent front).

generated by a phantom filled with known activity concentrations of either radionuclide. On the reconstructed images, regions of interest (ROIs) were drawn manually over the following regions using the Image Quantification and Kinetic Modeling Software PMOD 2.7: thoracic spine, lumbar spine, femur, humerus, bladder and abdomen. Furthermore, time–activity curves (TACs) in these six ROIs were generated. Tracer uptake was quantified as standardized uptake values (SUVs) using the following formula: SUV = Tissue Activity Concentration (Bq/cc)/Injected Dose (Bq) × Body Weight (g).

2.6. Biodistribution experiments

After the microPET scans, all animals were killed by cervical disruption; their organs (femurs, tail, liver, kidneys, lung, heart and spleen) were dissected within a few minutes, weighed and subjected to gamma counting. Percentage of injected dose per gram of tissue (%ID/g) was calculated using two calibration curves (high activity and low activity) with known activities and decay corrected for the injection time.

2.7. Statistical analyses

Statistical analyses were performed using the Microsoft Excel integrated analysis tool. Hypothesis tests among two data sets were made by comparison of two means from unpaired samples (independent t test). A P value lower than .05 was considered to be significant. Descriptive statistical analysis was performed using mean values and standard deviations.

3. Results

3.1. Radiotracer preparation

Complex formation was completed within 30 min, and radiochemical purity reliably exceeded 99% (Fig. 1).

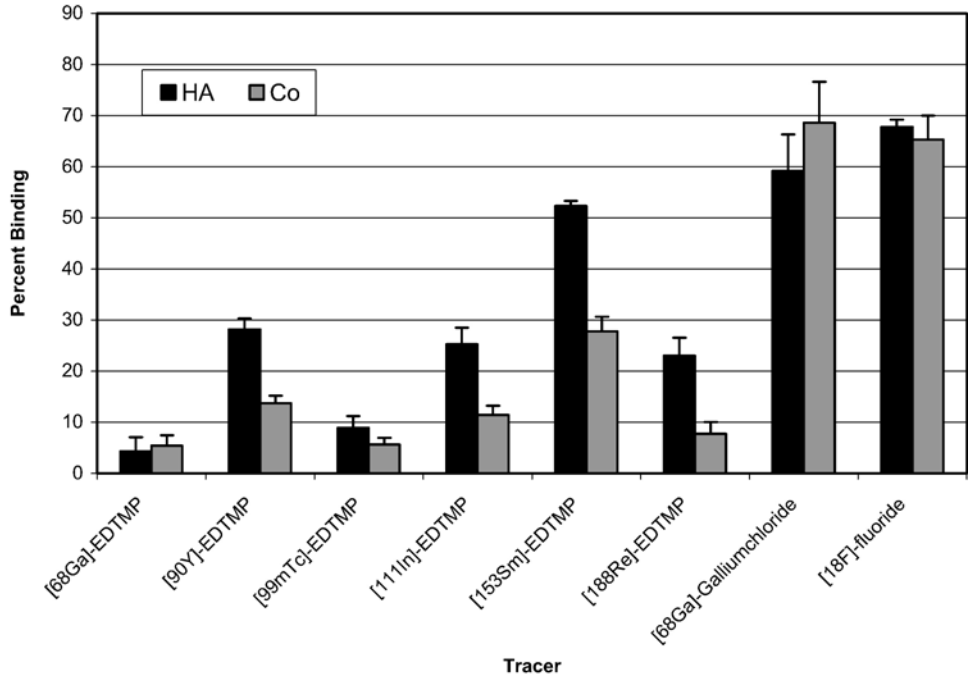

Fig. 2. Percentages of binding of various bone seekers on HA and Co (mean±S.D.). All values except those from [^{68}Ga]-EDTMP and [^{68}Ga]-gallium chloride are taken from References [9] and [10]. Each value represents the filter-corrected arithmetic mean of five experiments, with each measurement performed in triplicate.

3.2. Binding experiments (pre vivo model)

The results of the binding experiments are presented in Table 1. Values range from 1.08±1.89% for [^{68}Ga]-EDTMP on D-Co to 68.6±8.0% for [^{68}Ga]-gallium chloride on Co. Fig. 2 compares [^{68}Ga]-EDTMP with other ligand-based tracers as well as [^{18}F]-fluoride and [^{68}Ga]-gallium chloride. Statistical analyses — compared with [^{68}Ga]-EDTMP — revealed significantly higher binding values for all preparations except [^{99m}Tc]-EDTMP (both matrices) and [^{188}Re]-EDTMP (on Co).

3.3. MicroPET experiments

Representative PET images are shown in Fig. 3. The TACs of both tracers are presented in Fig. 4. The highest uptake was found in bladders, and uptake in osseous tissue was clearly visible. The reconstructed resolution for fluorine-18 was 1.3 mm of full width at half maximum (FWHM) in the center of the FOV (cFOV) and remained under 2 mm of FWHM within the central 5-cm-diameter FOV in all three dimensions. Due to the higher positron energy from gallium-68 (1.899 vs. 0.634 MeV for fluorine-18), the spatial resolution decreased to around 2 mm in the cFOV.

3.4. Biodistribution experiments

The results of biodistribution experiments are presented in Table 2. Bone uptake of [^{18}F]-fluoride was significantly higher than that of [^{68}Ga]-EDTMP ($P<.05$). The highest values were found in the bone and tail. No uptake of both tracers was found in the kidneys, lung, heart and spleen; additionally, no [^{68}Ga] activity uptake was found in the liver.

4. Discussion

The role of conventional [^{99m}Tc]-methylene diphosphonate scintigraphy as the standard of reference for detecting skeletal metastases from solid tumors or primary bone tumors [14,15] has recently been challenged by screening tools with similar or higher sensitivity levels, such as whole body magnetic resonance imaging, and those with higher sensitivity levels, such as [^{18}F]-FDG, [^{18}F]-fluoride PET, and combinations of these imaging techniques [16]. Unfortunately, [^{18}F]-FDG and [^{18}F]-fluoride are also discussed controversially due to their low diagnostic impact regarding sclerotic metastases and false-positive findings in minimal degenerative changes, respectively [4,5]. Thus, testing and introduction of new bone seekers for PET based on PPs might help clarify the pole position of skeletal scintigraphy in detecting bone lesions.

4.1. Radiotracer preparation

Convenient accessibility is a prerequisite for the broad acceptance of a new PET tracer. Even PET centers without a cyclotron should be able to independently prepare tracers for their clinical routine. As a major step toward this concept, a ^{68}Ge/^{68}Ga generator has been developed for the simple onsite preparation of [^{68}Ga]-gallium chloride.

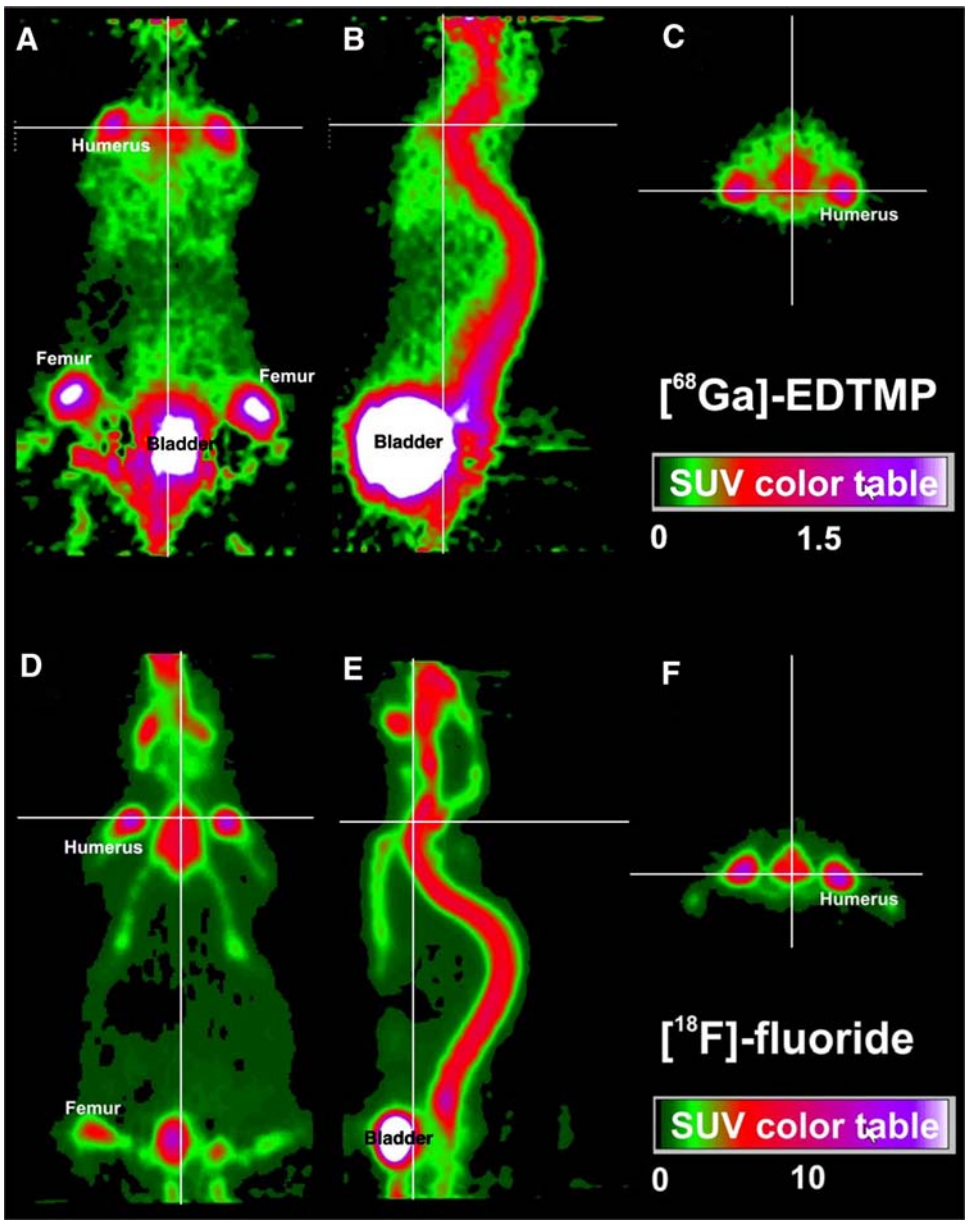

Fig. 3. Triplanar images of biodistribution using [^{68}Ga]-EDTMP (A, coronal; B, sagittal; C, axial) as well as those using [^{18}F]-fluoride (D, coronal; E, sagittal; F, axial) and microPET in two mice measured from 0 to 60 min. Corresponding areas are shown by white crosses. The color tables indicate the parametric SUVs of the summed frames. Injected doses were 2.05 and 4.17 MBq for [^{68}Ga]-EDTMP and [^{18}F]-fluoride, respectively.

In our study, [^{68}Ga]-gallium chloride was complexed with EDTMP using a commercially available kit system (Multibone) approved for the preparation of [^{99m}Tc]-EDTMP and [^{90}Y]-EDTMP. The complex formation was already complete after a reaction time of 5 min at room temperature and remained stable for at least 6 h, as checked with ascending ITLC (Fig. 1). Activity values of [^{68}Ga]-gallium were chosen, similar to previous experiments [9–11].

4.2. Binding experiments (pre vivo model)

For the evaluation of bone seekers, a pre vivo model was developed recently by our research group [9–11], which is similar to a method described earlier by Li et al. [12]. Thus, the binding of tracers on artificial and human bone allografts was determined by a specific filtration/gamma counting method. This method was based on the paradigm that bone seekers bind to the mineral phase of bone, which was finally supported by a subsequent study with osteoblasts [17]. In this pre vivo model, [^{68}Ga]-EDTMP showed very low binding, which was in contrast to the high binding of [^{68}Ga]-gallium chloride. Unfortunately, due to the known high protein-binding characteristics of [^{68}Ga]-gallium chloride, also supported by the high binding on D-Co (representing the organic compartment of bone), one cannot benefit from the advantage of drastically higher mineral binding in vivo.

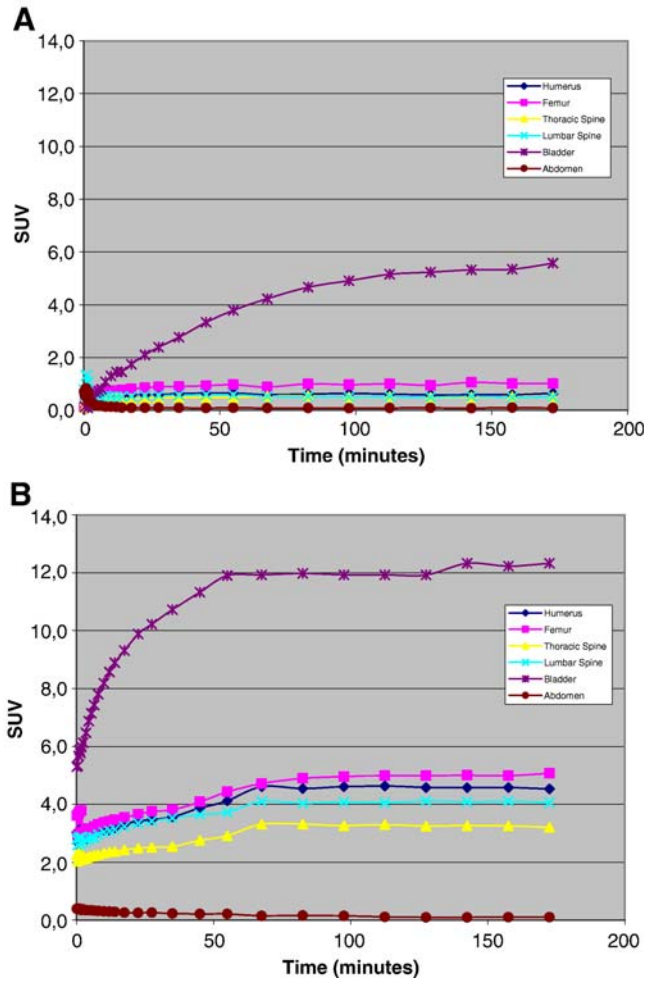

Fig. 4. (A) SUVs of [^{68}Ga]-EDTMP in various tissues (mean values of two mice). (B) SUVs of [^{18}F]-fluoride in various tissues (mean values of three mice).

Dewanjee et al. [7] stated that bone uptake of [^{68}Ga]-EDTMP is lower than that of [^{99m}Tc]-PPs, which is a finding reproduced by our model: [^{99m}Tc]-EDTMP binding was insignificantly higher and binding of [^{99m}Tc]-MDP as well as that of [^{99m}Tc]-DPD were significantly higher as compared with previously shown data [9,10]. In our series of evaluated bone seekers, [^{68}Ga]-EDTMP showed the lowest binding, whereas [^{18}F]-fluoride still appeared better.

4.3. MicroPET experiments

For a new PET tracer, prior to its application in humans, visualization of the major paths of its in vivo distribution is a prerequisite. The TACs and representative microPET images of [^{68}Ga]-EDTMP clearly show inferior skeletal accumulation as compared with [^{18}F]-fluoride. These findings are in line with the results of the pre vivo model and those of Dewanjee et al. [7]. In the microPET experiments, the image quality obtained with [^{68}Ga]-EDTMP was worse, which could be partially explained by the higher positron range of gallium-68 (see Results section). However, Yang et al. [18], in a study on the spatial resolution of human PET scanners, argued that under the assumption of at least a 3-mm spatial resolution of PET detectors, the conventional FWHM of fluorine-18 and that of gallium-68 are indistinguishable in soft tissue (3.01 vs. 3.09 mm). This implies that with the spatial resolution at 5–7 mm of current clinical scanners, the imaging quality using gallium-68-based tracers should be as good as that using fluorine-18-based agents [18].

4.4. Biodistribution experiments

Both evaluated bone seekers accumulated in the bone, whereas there was negligible uptake in soft tissue. The ex vivo experiments clearly showed that, overall, [^{18}F]-fluoride bone uptake was significantly higher than [^{68}Ga]-EDTMP uptake. Assuming that 12% of the total weight (mean=24.18 g) of mice is bone (2.9 g), the total uptake in the skeleton was 13.3% for [^{68}Ga]-EDTMP and was 38.6% for [^{18}F]-fluoride. Interestingly, using [^{68}Ga]-EDTMP, Dewanjee et al. [7] found 10%ID/g after 2 h and 21%ID/g after 3 h in ex vivo experiments with dogs as well as a bone-to-liver ratio of 15.8 and a bone-to-kidney ratio of 3.0 after 3 h [7]. Unfortunately, we could not calculate these ratios since there was no detectable uptake in the liver or kidney (Table 2). Since liver and kidney uptake could be explained by Phases I and II metabolism, lack of uptake in these organs could indicate a higher in vivo complex stability in our preparations. The high uptake values in the tail — showing good correlation with femur — could be explained by binding to the tailbone.

5. Conclusions

From the present experiments, it is evident that [^{68}Ga]-EDTMP is accessible via simple kit preparation and predominantly accumulated in bone tissue in vivo, ex vivo and pre vivo. Binding to mineral bone was irreversible, and very low binding was observed in organic bone tissue. Compared with [^{18}F]-fluoride, the uptake was lower and the PET image quality was reduced. The binding superiority of [^{18}F]-fluoride was coherently demonstrated throughout the whole study — in vivo, ex vivo and pre vivo. From the present evaluation, apart from the autonomy for PET centers without an onsite cyclotron, the advantage of [^{68}Ga]-

Table 2
Values counted in various organs after the injection of [^{18}F]-fluoride or [^{68}Ga]-EDTMP as %ID/g ($n>3$)

Organ	[^{18}F]-fluoride		[^{68}Ga]-EDTMP	
	%ID/g	S.D.	%ID/g	S.D.
Femur	13.3	2.79	4.60	1.18
Tail	4.68	1.85	4.68	4.84
Liver	0.03	0.07	<0.01	–
Kidneys	<0.01	–	<0.01	–
Lung	<0.01	–	<0.01	–
Heart	<0.01	–	<0.01	–
Spleen	<0.01	–	<0.01	–

EDTMP over [^{18}F]-fluoride is not apparent and the future clinical prospect of [^{68}Ga]-EDTMP remains speculative.

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