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Research paper

Preparation and pre-vivo evaluation of no-carrier-added, carrier-added and cross-complexed [^{68}Ga]-EDTMP formulations

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Abstract

Purpose: The present study aimed to develop convenient preparation and quality control protocols for [^{68}Ga]-EDTMP, a potential radiotracer for skeletal PET imaging. Furthermore, bone binding characteristics with special focus on the influence of carrier addition were evaluated.

Methods: No-carrier-added (nca), carrier-added and novel cross-complexed [^{68}Ga]-EDTMP formulations were prepared using [^{68}Ga]-gallium chloride and a commercial EDTMP kit. Respective bone binding characteristics were determined on the basis of an established in-vitro method using hydroxyapatite and human bone powders as binding matrices.

Results: Pre-vivo evaluation of nca [^{68}Ga]-EDTMP yielded irreversible binding on the mineral bone phase characterised by fast binding kinetics. Generally, nca [^{68}Ga]-EDTMP showed low uptake values comparable to nca [^{99m}Tc]-EDTMP. Interestingly, the bone binding affinity of [^{68}Ga]-EDTMP could be increased by the addition of carriers, presumably by changing the complex structure.

Conclusions: This fast and reliable preparation protocol could enable small PET facilities without onsite cyclotron to perform PET bone scans. A comparison of all cross-complexed [^{68}Ga]-EDTMP preparations further strengthens the recently presented ''foreign carrier theory'', which highlights carrier addition as a factor strongly affecting bone uptake of radiolabelled polyphosphonates. The clinical applicability of [^{68}Ga]-EDTMP – particularly with respect to lesion specificity and sensitivity – should be clarified in forthcoming in-vivo studies.

Keywords: Gallium-68; EDTMP; PET; Bone imaging; Carrier; Targeting; Pharmacokinetics; Binding studies

1. Introduction

Since many prevalent tumours are associated with the occurrence of bone metastases, skeletal imaging and bone pain palliation represent major tasks in clinical nuclear medicine. Minimization of both the radiation burden to the patient and interfering signals in the scintigraphic image requires specific skeletal localization of the radiopharmaceuticals [1]. In order to target a chosen radionuclide into bone tissue, polyphosphonates (PP) such as MDP (methylene diphosphonate), DPD (3,3-diphosphono-1,2-propandicarboxylic acid) and EDTMP (ethylenediamino-*N*,*N*,*N*′,*N*′-tetrakis-methylene-phosphoric acid) have been established as suitable ligands [2]. In this approach, bone localization is a property of the phosphorous compound while the radioactivity is associated with

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a radionuclide bound to the PP within a complex. Depending on its application for diagnosis or therapy, the radionuclide is chosen according to its physical properties.

Although PP have been used for more than 30 years in nuclear medicine, there is still controversy about the underlying mechanisms involved in their uptake into bone [3–5]. In recent studies, the authors presented a method for the pre-vivo evaluation of bone seekers involving binding studies of radiotracer formulations on artificially manufactured bone compartments and human bone powders [6,7]. Using this model, the uptake of radiolabelled bone seekers could be defined as an irreversible process on bone forming minerals with minor contribution of the organic phase of bone including osteoblasts [8]. A variety of factors such as the choice of the radionuclide, the ligand concentration, the preparation method and cross-complexation were shown to influence the uptake behaviour of PP.

The term cross-complexation describes the addition of a stable nuclide to a radioactive complex preparation. This so-called carrier can either be ''related'' or ''foreign'' to the radionuclide, depending on their physical–chemical properties like electron configuration, electronegativity and ionic volume. In-vitro and in-vivo experiments revealed that the binding capacity of PP could be significantly increased by cross-complexation, e.g. by adding stable rhenium isotopes to a [^{99m}Tc]-EDTMP preparation [6,9,10]. Interestingly, evidence has been provided that the more foreign a carrier of a cross-complexed preparation is, the more binding enhancement on human bone matrices can be achieved in-vitro. This observation resulted in the presentation of a novel ''foreign carrier theory'' [11].

Currently, [^{99m}Tc]-MDP represents the working horse for diagnostic bone scanning in conventional nuclear medicine. However, positron emission tomography (PET) is increasingly gaining interest as a powerful alternative providing improved spatial resolution and lesion contrast [12]. Both cyclotron produced [^{18}F]-fluoride – as a non-specific bone tracer – and [^{18}F]-fluorodeoxyglucose (^{18}FDG) – imaging altered tumour metabolism – have been described as bone imaging agents in clinical studies [13–16]. Another radionuclide suitable for PET imaging is gallium-68 with a short half life of 68 min. As a group IIIa element, gallium further provides the possibility to form complexes with suitable ligands. [^{68}Ga]-EDTMP has been first described in 1976 and bioevaluation in dogs brought evidence for its clinical usefulness as bone localizing agent [17]. Nevertheless, [^{68}Ga]-EDTMP did not find its way to clinical application, presumably due to the following reasons: (1) The 511 keV radiation arising from the positron annihilation has an energy which is above the optimum energy for conventional imaging devices, (2) PET scanners were not commonly available for bone scanning, (3) the advent of technetium-99m labelled PP represented strong competition, and (4) the ^{68}Ge/^{68}Ga generators of the first generation yielded gallium-68 in the form of an EDTA complex calling for sophisticated chemical manipulation.

Nowadays, due to the rising number of modern PET scanners and a commercially available second generation of ^{68}Ge/^{68}Ga generators yielding [^{68}Ga]-gallium chloride, [^{68}Ga]-EDTMP could represent a convenient agent for PET bone scanning in PET centres without onsite cyclotron.

The present study aimed to develop preparation and quality control protocols for no-carrier-added (nca), carrier-added (ca) and novel cross-complexed [^{68}Ga]-EDTMP formulations using a commercially available EDTMP kit system. Furthermore, the respective bone binding characteristics were evaluated on the basis of our established in-vitro method using hydroxyapatite and human bone powders as binding matrices. Particular focus was set on the influence of carrier addition on bone binding capacities aiming at the verification of the recently introduced ''foreign carrier theory''.

2. Materials and methods

2.1. Materials

Multibone® kits were commercially obtained (Izotop, Budapest, Hungary). Sterile phys. saline (0.154 M) was from Fresenius Kabi (Graz, Austria). Perrhenic acid (70% aqueous), indium chloride (98%), gallium chloride (99.9%), Hanks' balanced salt solution (HBSS) (H 8264), HA (21223) and yttrium chloride hexahydrate (>99.9%) were purchased from Sigma–Aldrich (Steinheim, Germany). The Elutec [^{99}Mo/^{99m}Tc]-generator was obtained from Bristol-Myers Squibb (Brussels, Belgium). Millex-SG 0.22-μm sterile filters were from Millipore (Bedford, MA, USA). Measurements of radioactivity were performed on a Cobra II auto-gamma-counter (Canberra Packard, Canada). The thermostatic water bath 1083 was from GFL (Burgwedel, Germany), and the dose calibrator was a Curiementor 2 from PTW (Freiburg, Germany). Particle size analyses were performed using a SALD-1100 (Shimadzu, Japan). The 1110 MBq ^{68}Ge/^{68}Ge generator was obtained from I.D.B Holland B.V. (Baarle-Nassau, Netherlands). ITLC/SG strips were from Gelman (Ann Arbor, MI, USA), auto-radiography was performed on an Instant Imager (Canberra Packard, Pangbourne, UK).

2.2. Preparation of human cortical bone powder and demineralised human cortical bone powder

The preparation of binding matrices followed the methods reported previously [7]. Briefly, human bone specimens were excised from donors, washed, freeze-dried and sterilized. Afterwards, these cortical allografts were processed into a fine powder by milling (Co). For the preparation of demineralised human bone powder (D-Co), bone allografts were decalcified using 1% hydrochloric acid prior to milling. Measurements revealed a particle size in a

comparable range to commercially available hydroxyapatite (HA).

2.3. Radiotracer preparation

For the preparation of [^{68}Ga]-containing radiotracers, the ^{68}Ge/^{68}Ga generator was eluted with 5 ml of 1 M hydrochloric acid (330–520 MBq). Afterwards, 2 ml of this eluate was diluted with 3 ml phys. saline to provide a solution of [^{68}Ga]-gallium chloride in a volume of 5 ml.

[^{68}Ga]-EDTMP: 5 ml of [^{68}Ga]-gallium chloride solution was added to a Multibone® kit and kept at ambient temperature for 30 min.

[^{68}Ga]-EDTMP with gallium or yttrium carrier: 1.75 mg of gallium chloride or 3 mg yttrium chloride was added to a Multibone® kit prior to the addition of 5 ml [^{68}Ga]-gallium chloride solution. The resulting mixture (2 mM carrier) was kept for 30 min at ambient temperature.

[^{68}Ga]-EDTMP with indium carrier: 2.21 mg of indium chloride was added to a Multibone® kit prior to the addition of 5 ml [^{68}Ga]-gallium chloride solution. The resulting mixture (2 mM carrier) was kept for 30 min at ambient temperature and filtered through a Millipore filter unit (0.22 μm) to remove indium colloid.

[^{68}Ga]-EDTMP with rhenium carrier: 15 μl (87 μmol) of perrhenate was added to 5 ml [^{68}Ga]-gallium chloride solution. Tin chloride (3.5 mg) was added to the Multibone® kit prior to incubation with the [^{68}Ga]-gallium chloride solution at ambient temperature for 30 min.

2.4. Quality control

Complex integrity of all [^{68}Ga]-EDTMP preparations was checked with ascending ITLC/SG 1×8 cm strips developed in 1:1 methanol/ammonium acetate.

2.5. Binding experiments

To a vial containing 3 mg of HA, Co or D-Co 3 ml of HBSS was added and the vial was swayed at 37 °C for 24 h. Radioactive-labelled PP (content of ligand: 0.3 μmol) or 25 MBq [^{68}Ga]-gallium chloride was added, the tube was replaced in the water bath (120 min, 37 °C) and vortexed every 15 min and before extraction. Kinetic studies of nca [^{68}Ga]-EDTMP were performed after 15, 30, 60, 120 and 240 min incubation time.

2.6. Binding measurement

An aliquot of 50 μl of this suspension was added to 2 ml of phys. saline. Out of this dilution, three aliquots of 50 μl were taken and placed in tubes for the gamma-counter. The rest of the dilution was filtered through a Millex-SG single use filter unit, and three aliquots of 50 μl were taken from the filtrate and placed in tubes. The radioactivity of the six tubes was measured in the gamma-counter, and the percentage of irreversibly bound radiolabelled PP was calculated as percent binding.

2.7. Assessment of binding reversibility

After proceeding a binding experiment with [^{68}Ga]-gallium chloride solution and [^{68}Ga]-EDTMP on 3 mg HA, Co and D-Co following the described procedure vide supra, an aliquot was taken from the remaining suspension and diluted at a ratio of $1 + 1$ and $1 + 4$ with HBSS. The diluted samples were replaced in the water bath for another 120 min. The following work up procedure was the same as described above.

2.8. Filter experiments and filter correction

Filter experiments were performed to control for the amount of radioactivity retained unspecifically during filtration. The procedure was similar to the binding experiments and binding measurement. The only modification was the omission of binding matrix and associated incubation periods. The blank values obtained from filter experiments were converted iteratively [6] and subtracted from the binding values derived from the binding measurements to obtain filter corrected data.

2.9. Statistics

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 independent (unpaired) samples (*t*-test). A value of $p < 0.05$ was considered significant. Descriptive statistical analyses were performed using mean values and standard deviations.

3. Results

3.1. Radiotracer preparation

The complex formation of gallium-68 and EDTMP was completed after 5 min reaction time at room temperature and remained stable for at least 5 h (data not shown). Illustrative images showing the principle of the ITLC assay are presented in Fig. 1. Using 1:1 methanol/ammonium acetate for ITLC development allowed the detection of free gallium-68 ions which remained at the starting point (Fig. 1a), whereas [^{68}Ga]-EDTMP ascended with the front (Fig. 1b). Radiochemical purity of the nca [^{68}Ga]-EDTMP product reliably exceeded 98%. Carrier-added and cross-complexed [^{68}Ga]-EDTMP formulations were prepared by a simple modification of the Multibone® kit. For that purpose, stable gallium, yttrium, indium or rhenium compounds were added to the reaction mixture prior to the addition of the gallium eluate. The resulting complexes were quality controlled after 30 min reaction time and

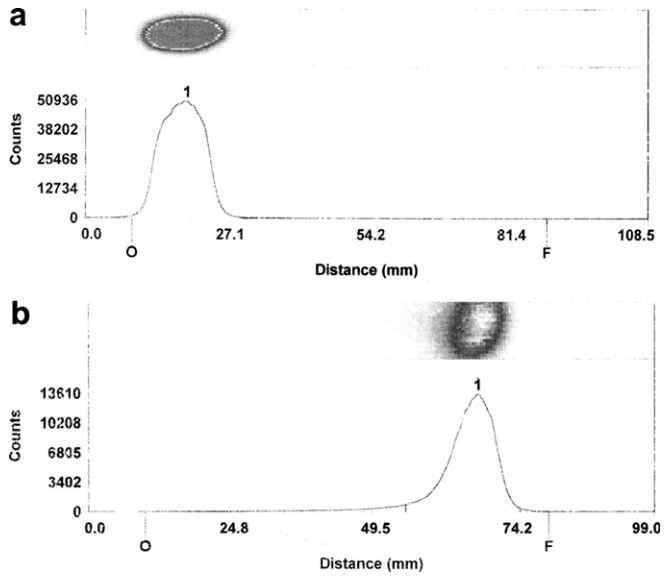

Fig. 1. The principle of the ITLC assay applied for the quality control of [^{68}Ga]-EDTMP preparations. Using 1:1 methanol/ammonium acetate development, [^{68}Ga]-gallium chloride remained at the starting point (a), whereas the [^{68}Ga]-EDTMP complexes did migrate with the solvent front (b).

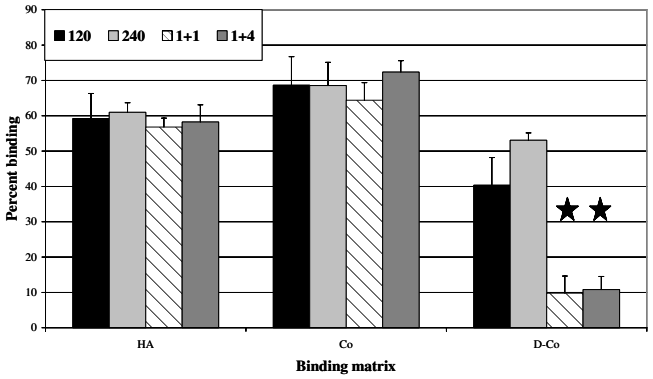

Fig. 2. Binding of 25 MBq [^{68}Ga]-gallium chloride solution on 3 mg hydroxyapatite (HA), human cortical matrix (Co) and demineralised bone matrix (D-Co) after 120 and 240 min incubation time. Additionally, results of the binding reversibility experiments are shown. After an incubation period of 120 min, the incubation mixture was diluted at a ratio of both 1 + 1 and 1 + 4 and incubated for another 120 min. Each value represents the filter-corrected arithmetic mean of five experiments with each measurement performed in triplicate. Error bars represent the standard deviation from the mean. Significant differences ($p < 0.01$) from the respective binding value after 240 min incubation time are marked with an asterisk.

radiochemical purity of >94% was achieved in each case as evaluated using the described quality control assay.

3.2. Pre-vivo evaluation of bone seekers

Prior to the binding assay, filter experiments were performed in order to determine the amount of ^{68}Ga activity lost by the filtering process. Values shown in Table 1 were subtracted iteratively to yield the corrected binding values of the radiotracers presented in Figs. 2, 3 and Table 2.

Fig. 2 presents the binding values of [^{68}Ga]-gallium chloride on HA, Co and D-Co. After 120 min incubation time [^{68}Ga]-gallium chloride yielded $59.2 \pm 7.1\%$ and $68.6 \pm 8.1\%$ on the mineral bone matrices HA and Co, respectively. Binding reversibility studies showed that the uptake of [^{68}Ga]-gallium chloride on mineral matrices represents an irreversible process since no significant drop in binding values was observable after dilution and washing procedures [6,7]. In contrast, binding values on D-Co yielded a significant decrease ($p < 0.05$) in the reversibility experiments (Fig. 2). Table 2 shows the binding kinetics

Table 1
The percent binding of the evaluated tracers retained on the filter

Radiotracer	Mean ± SD
[^{68}Ga]-Gallium chloride solution	4.9 ± 1.5
[^{68}Ga]-EDTMP	5.6 ± 1.5
[^{68}Ga]-/Ga-EDTMP	3.4 ± 1.5
[^{68}Ga]-/In-EDTMP	7.2 ± 4.2
[^{68}Ga]-/Y-EDTMP	2.0 ± 1.3
[^{68}Ga]-/Re-EDTMP	1.6 ± 1.2

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

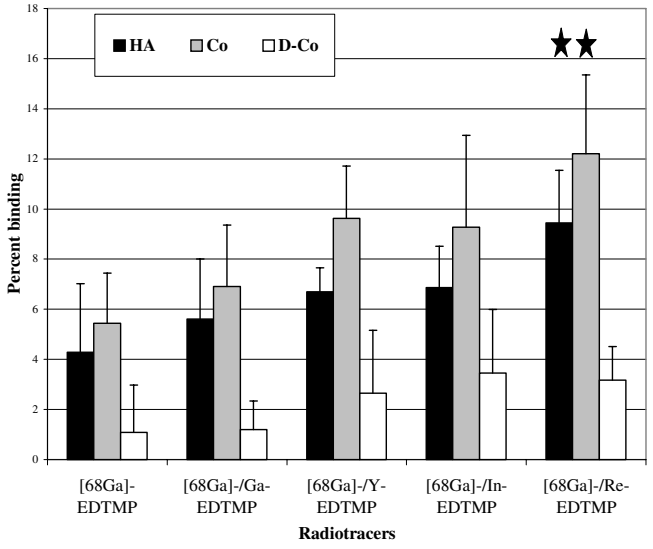

Fig. 3. The binding of no-carrier-added and carrier-added [^{68}Ga]-EDTMP on 3 mg hydroxyapatite (HA), human cortical matrix (Co) and demineralised bone matrix (D-Co) in percent. Each value represents the filter-corrected arithmetic mean of five experiments with each measurement performed in triplicate. Error bars represent the standard deviation from the mean. Significant differences from no-carrier-added [^{68}Ga]-EDTMP values ($p < 0.05$) are marked with an asterisk.

of nca [^{68}Ga]-EDTMP on HA, Co and D-Co. The results indicate that the uptake of nca [^{68}Ga]-EDTMP on the mineral matrices was finished after 15 min since no significant increase of the binding capacity was observable after longer incubation times.

A comparison of all cross-complexed [^{68}Ga]-EDTMP formulations is displayed in Fig. 3. Interestingly, the binding values of the respective cross-complexed [^{68}Ga]-EDTMP preparations changed as a function of carrier

Table 2
Binding kinetics of nca [^{68}Ga]-EDTMP on 3 mg of hydroxyapatite (HA) and human cortical matrix (Co) and demineralised bone matrix (D-Co)

Incubation time (min)	Percent binding on matrix (mean ± SD)		
	HA	Co	D-Co
15	3.53 ± 0.52	5.67 ± 3.21	
30	2.40 ± 2.77	5.56 ± 3.23	
60	3.28 ± 2.77	4.31 ± 3.21	
120	4.29 ± 2.74	5.44 ± 1.99	1.08 ± 1.89
240	4.89 ± 2.14	5.73 ± 2.45	1.17 ± 0.79
240 (1 + 4 dilution)	4.57 ± 2.17	5.19 ± 3.53	0.13 ± 1.58

Additionally, results of the binding reversibility experiments are shown. After an incubation period of 120 min, the incubation mixture was diluted at a ratio of 1 + 4 and incubated for another 120 min. Each value represents the filter-corrected arithmetic mean of five experiments with each measurement performed in triplicate.

addition, resulting in significantly higher binding values of [^{68}Ga]-/Re-EDTMP on HA and Co as compared to the nca formulation ($p = 0.001$ and 0.017, respectively).

4. Discussion

4.1. Radiotracer preparation

Positron emitting radionuclides such as [^{18}F]-fluoride and [^{68}Ga]-gallium chloride have been firstly applied for skeletal imaging in the 1960s [18,19]. Both [^{18}F]-fluoride and [^{68}Ga]-gallium chloride preferentially accumulate in metabolically active regions of bone that undergo accelerated resorption and formation [1,20]. One main drawback of using [^{68}Ga]-gallium chloride as a bone imaging agent lies in its remarkable protein binding capacity [1]. For intra-venous application, [^{68}Ga]-gallium chloride had therefore to be coupled with appropriate amounts of gallium carrier to reduce the general background of the images by saturating the preferred binding sites in serum proteins. The need to suppress the protein binding capacity of gallium led to the exploration of using PP ligands, initially developed for technetium-99m, as bone localizing chelates for gallium-68 [17]. In this single study on [^{68}Ga]-EDTMP, the ^{68}Ga activity was eluted from a ^{68}Ge/^{68}Ga generator as a stable EDTA complex and time-consuming exchange for EDTMP had to be applied.

In the present study, the authors presented a protocol for the convenient preparation of nca [^{68}Ga]-EDTMP using [^{68}Ga]-gallium chloride and a commercially available kit system (Multibone®). This kit is approved for the preparation of [^{99m}Tc]-EDTMP and [^{90}Y]-EDTMP for human application. Since both gallium and yttrium are group III elements with related characteristics, the authors hypothesized the use of this kit for the production of nca [^{68}Ga]-EDTMP. Convenient availability of a radiotracer is a prerequisite for its acceptance in clinical routine application. Therefore, the presented fast and reliable preparation method for nca [^{68}Ga]-EDTMP could facilitate an alternative to cyclotron-dependent [^{18}F]-fluoride PET bone scanning.

4.2. Pre-vivo evaluation of bone seekers

For the evaluation of the binding characteristics of gallium-containing bone seekers, the authors applied a previously introduced in-vitro method. This method is based on the measurement of the percent binding of radiotracers on synthetic and human bone matrices by a simple filtration process followed by gamma counting [6,7].

In this pre-vivo model, [^{68}Ga]-gallium chloride showed high binding affinity comparable to that of [^{18}F]-fluoride which was evaluated recently using the same model [6,7]. However, the high binding to the organic phase of bone (D-Co) together with the high degree of binding reversibility – reflects the remarkable extent of unspecific binding of [^{68}Ga]-gallium chloride. Unfortunately, due to its protein binding characteristics, in-vivo studies with [^{68}Ga]-gallium chloride cannot benefit from its pronounced mineral binding capacity. To overcome protein binding of free gallium ions in vivo, gallium-68 containing PP complexes were prepared and discussed in the literature [1,17]. Based on our pre-vivo model, we evaluated [^{68}Ga]-EDTMP using both artificial and human bone minerals. The results of the present study document fast uptake kinetics and irreversible binding of nca [^{68}Ga]-EDTMP on HA and Co. Interestingly, the binding values of nca [^{68}Ga]-EDTMP are generally very low. The incorporation of ^{68}Ga into the EDTMP complex apparently reduced the high binding affinity of gallium-68 ions to mineral bone matrices about 13-fold in our model. Compared to other EDTMP preparations – evaluated in previous studies using the same method [6,7] – nca [^{68}Ga]-EDTMP represents the tracer with the lowest affinity to mineral matrices. On Co, binding of nca [^{68}Ga]-EDTMP was 1.1-, 2.1-, 2.5- and 5.1-fold lower than nca [^{99m}Tc]-EDTMP, [^{111}In]-EDTMP, [^{90}Y]-EDTMP, and [^{153}Sm]-EDTMP, respectively. This finding clearly demonstrates the remarkable influence of the radionuclide on the binding characteristics of ligand-based bone seeking agents.

The presented in-vitro method is a rapid and simple way to examine the adsorption of radioactive-labelled substances on bone components and correlations with published in-vivo data support its applicability as a model for the evaluation of parameters influencing tracer–matrix interactions [6,7,11]. However, it is important to consider that the in-vivo uptake of radiotracers into bone is embossed by a variety of factors hardly reproducible by any model. Particularly, the distribution of bone seekers within osseous tissue, i.e. the specificity for pathological changes such as metastases or inflammations, cannot be predicted. Therefore, PET bone scanning using [^{68}Ga]-EDTMP should be evaluated and compared to [^{18}F]-fluoride with respect to in-vivo bone uptake, bone-to-soft-tissue-ratio, lesion-to-normal-bone-ratio, as well as sensitivity and specificity for bone lesions. This in-vivo

evaluation, however, is beyond the scope of the present report and should be addressed in forthcoming studies.

4.3. Influence of carrier addition on bone binding affinity of [^{68}Ga]-EDTMP

The concept of increasing the bone binding affinity of radiotracers by cross-complexation has been outlined by the authors both in vitro [6,7,11] and in vivo [9,10] using different EDTMP-based bone seekers. Based on this concept, a further aim of the present study was to investigate the possibility to increase the in-vitro bone binding capacity of nca [^{68}Ga]-EDTMP by the formation of cross-complexes. Therefore, binding experiments of [^{68}Ga]-/Ga-EDTMP, [^{68}Ga]-/Y-EDTMP [^{68}Ga]-/In-EDTMP, and [^{68}Ga]-/Re-EDTMP on HA, Co and D-Co were performed following the described procedure. The results displayed in Fig. 3 clearly support the "foreign carrier theory" as previously presented by the authors [11]. Because the electron configuration is of great importance for the coordination sphere of a complex, yttrium and indium are closer related to gallium (all three valence electrons, related) than to Re (3 vs. 7 valence electrons; foreign). Therefore, regarding gallium as reference radionuclide, the following carriers would become more foreign in the order: gallium < yttrium ≈ indium < rhenium. We found that the binding values of the respective cross-complexed [^{68}Ga]-EDTMP preparations increased in exactly the same order, resulting in significantly higher binding values of [^{68}Ga]-/Re-EDTMP on HA and Co. This influence of carrier addition as well as the general influence of the radionuclide on binding characteristics could be explained by the rearrangement of complex structures or formation of polymeric structures initiated by the carriers and the radionuclides themselves [21] resulting in elevated bone binding affinity.

5. Conclusion

The presented study describes the fast and convenient preparation of nca [^{68}Ga]-EDTMP with high radiochemical purity, enabling small PET facilities without onsite cyclotron to perform PET bone scans. Pre-vivo evaluation of bone binding capacities resulted in low uptake values in the range of nca [^{99m}Tc]-EDTMP which, however, could be increased by the addition of carriers, presumably by changing the complex structure. A comparison of all cross-complexed [^{68}Ga]-EDTMP preparations further strengthens the recently presented "foreign carrier theory", which highlights carrier addition as an important factor strongly affecting bone binding characteristics of radiolabelled PP complexes. The clinical applicability of [^{68}Ga]-EDTMP – particularly with respect to lesion specificity and cost-effectiveness compared to [^{18}F]-fluoride PET bone scans – should be addressed in forthcoming in-vivo studies.

References

[1] M.W. Billinghurst, Radio ion exchange in bone, in: M.W. Billinghurst, L.G. Colombetti (Eds.), Studies of Cellular Function Using Radiotracers, CRC Press Inc., Florida, 1982, pp. 93–114.

[2] H.M. Chilton, M.D. Francis, J.H. Thrall, Radiopharmaceuticals for bone and bone marrow imaging, in: D.P. Swanson, H.M. Chilton, J.H. Thrall (Eds.), Pharmaceuticals in Medical Imaging, Macmillan, New York, 1990, pp. 537–563.

[3] M.D. Francis, I. Fogelman, [99mTc] Diphosphonate Uptake Mechanisms on Bone. Bone Scanning in Clinical Practice, Springer, London, 1987.

[4] D. Kanishi, 99mTc-MDP accumulation mechanisms in bone, Oral Surg., Oral Med. Oral Pathol. 75 (1993) 239–246.

[5] Z. Schwartz, J. Shani, W.A. Soskolne, H. Touma, D. Amir, J. Sela, Uptake and biodistribution of technetium-99m–MD32P during rat tibial bone repair, J. Nucl. Med. 34 (1993) 104–108.

[6] M. Mitterhauser, S. Togel, W. Wadsak, L.K. Mien, H. Eidherr, K. Wiesner, V. Viernstein, K. Kletter, R. Dudczak, Binding Studies of [^{18}F]-fluoride and polyphosphonates radiolabelled with [^{111}In], [^{99m}Tc], [^{153}Sm] and [^{188}Re] on bone compartments: a new model for the pre vivo-evaluation of bone seekers? Bone 34 (2004) 835–844.

[7] M. Mitterhauser, S. Toegel, W. Wadsak, L.K. Mien, H. Eidherr, K. Kletter, H. Viernstein, R. Kluger, A. Engel, R. Dudczak, Binding studies of [^{18}F]-fluoride and polyphosphonates radiolabelled with [^{99m}Tc], [^{111}In], [^{153}Sm] and [^{188}Re] on bone compartments: verification of the pre-vivo model? Bone 37 (2005) 404–412.

[8] S. Toegel, O. Hoffmann, W. Wadsak, D. Ettlinger, L.K. Mien, K. Wiesner, J. Nguemo, H. Viernstein, K. Kletter, R. Dudczak, M. Mitterhauser, Uptake of boneseekers is solely associated with mineralisation! a study with [99mTc]-MDP, [153Sm]-EDTMP and [^{18}F]-fluoride on osteoblasts, Eur. J. Nucl. Med. Mol. Imaging 33 (2006) 491–494.

[9] M. Mitterhauser, A. Krcal, R. Dudczak, T. Traub, S. Oflouglu, H. Viernstein, C. Pirich, Synthesis of 99mTc EDTMP using perrhenic acid as carrier – radiopharmaceutical and clinical results, Eur. J. Nucl. Med. Mol. Imaging 28 (2001) 1028.

[10] B. Fuger, M. Mitterhauser, W. Wadsak, S. Ofluoglu, T. Traub, G. Karanikas, R. Dudczak, C. Pirich, Bone lesion detection with carrier added Tc-99m EDTMP in comparison to Tc-99m DPD, Nucl. Med. Commun. 25 (2004) 361–365.

[11] S. Toegel, L.K. Mien, W. Wadsak, H. Eidherr, H. Viernstein, R. Kluger, K. Kletter, R. Dudczak, M. Mitterhauser, In-vitro evaluation of nca., ca. and cross-complexed [^{90}Y]-EDTMP provides evidence for a novel "foreign carrier theory", Nucl. Med. Biol. 33 (2006) 95–99.

[12] G.J.R. Cook, I. Fogelman, Detection of bone metastases in cancer patients by [^{18}F]-fluoride and [^{18}F]-fluorodeoxyglucose positron emission tomography, Q. J. Nucl. Med. 45 (2001) 47–52.

[13] M. Hetzel, C. Arslandemir, H.H. Konig, A.K. Buck, K. Nussle, G. Glattnig, A. Gabelmann, J. Hetzel, V. Hombach, H. Schirrmeister, [^{18}F] NaF PET for detection of bone metastases in lung cancer: accuracy, cost-effectiveness, and impact on patient management, J. Bone Miner. Res. 18 (2003) 2206–2214.

[14] H. Schirrmeister, A. Guhlmann, J. Kotzerke, C. Santjohanser, T. Kuhn, R. Kreienberg, P. Messer, K. Nussle, K. Elsner, G. Glatting, H. Trager, B. Neumaier, C. Diederichs, S.N. Reske, Early detection and accurate description of extent of metastatic bone disease in breast cancer with fluoride ion and positron emission tomography, J. Clin. Oncol. 17 (1999) 2381–2389.

[15] G.J. Cook, S. Houston, R. Rubens, M.N. Maisey, I. Fogelman, Detection of bone metastases in breast cancer by ^{18}FDG PET: differing metabolic activity in osteoblastic and osteolytic lesions, J. Clin. Oncol. 16 (1998) 3375–3379.

[16] E. Even-Sapir, U. Metser, G. Flusser, L. Zuriel, Y. Kollender, H. Lerman, G. Lievshitz, I. Ron, E. Mishani, Assessment of malignant skeletal disease: initial experience with [^{18}F]-fluoride PET/CT and

comparison between [^{18}F]-fluoride PET and [^{18}F]-fluoride PET/CT, J. Nucl. Med. 45 (2004) 272–278.
[17] M.K. Dewanjee, D.J. Hnatowich, R. Beh, New 68Ga-labeled skeletal-imaging agents for positron scintigraphy, J. Nucl. Med. 17 (1976) 1003–1007.
[18] R.L. Hayes, J.E. Carlton, B.L. Byrd, Bone scanning with gallium-68: a carrier effect, J. Nucl. Med. 6 (1965) 605–610.
[19] D.A. Weber, E.J. Greenberg, A. Dimich, P.J. Kenny, E.O. Rothschild, W.P. Myers, J.S. Laughlin, Kinetics of radionuclides used for bone studies, J. Nucl. Med. 10 (1969) 8–17.
[20] R.P. Warrell, Gallium for treatment of bone diseases, in: G. Berthon (Ed.), Handbook of Metal–Ligand Interactions in Biological Fluids, vol. 2, M. Dekker, New York, 1995, pp. 1253–1265.
[21] R.C. Elder, J. Yuan, B. Helmer, D. Pipes, K. Deutsch, E. Deutsch, Studies of the structure and composition of rhenium-1,1-hydroxyethylidenediphosphonate (HEDP). Analogues of the radiotherapeutic agent ^{186}ReHEDP, Inorg. Chem. 36 (1997) 3055–3063.