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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?

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Abstract

Introduction: Although the first polyphosphonates (PP) were introduced to nuclear medicine as bone imagers in the early 70s, mechanisms involved in uptake still remain speculative. Controversies range from adsorption onto the mineral phase with disputed binding to the organic phase, over incorporation into the mineralisation process to a combination of both mechanisms. Other factors such as solubility of the complex, concentration of ligand or effects of the radionuclide have also been discussed as possible parameters influencing bone uptake. Therefore, the present work aimed to verify the recently presented pre vivo model which was developed to rate the influence of various factors on the binding of differently radiolabelled PP and [^{18}F]-fluoride on synthetic bone matrix.

Methods: Radiolabelled polyphosphonates and [^{18}F]-fluoride were added to a vial containing lyophilised and milled spongiosa (Sp) or cortical bone (Co) in Hank's Balanced Salt Solution. After incubation, the radioactivity was measured in the gamma-counter before and after filtration. The percentage of irreversibly bound radioactivity was calculated. Same experiments were performed after decalcification of Sp and Co with hydrochloric acid.

Results: Descriptively, [^{111}In] increases the uptake of EDTMP in each case compared to similarly prepared [^{99m}Tc]-analogues: [^{111}In]-EDTMP > [^{99m}Tc]-EDTMP, [^{111}In]-/In-EDTMP > [^{99m}Tc]-/In-EDTMP and [^{111}In]-/Re-EDTMP > [^{99m}Tc]-/Re-EDTMP. [^{188}Re]-EDTMP shows higher binding than the carrier-added analogue, contradicting recent in vivo findings of [^{188}Re]-PP. However, our findings on human matrix are consistent with those of a previous study using artificial bone material. Binding on decalcified tissue was very low (PP) to moderate ([^{18}F]-fluoride) and reversible. Remarkable is also the unrivalled high uptake of [^{18}F]-fluoride, showing no reduced uptake on Co and Sp as compared to hydroxyapatite (HA) and amorphous calcium phosphate (ACP).

Conclusion: The binding of the evaluated bone seekers on these human bone matrices follows a comparable pattern as on artificial bone. The present study substantiates the fact that binding predominantly occurs on the inorganic compartment of bone. The best correlation was found between HA and Co. Therefore, HA can serve as a matrix for representative binding studies.

Keywords: Polyphosphonates; Binding studies; Technetium-99m; Fluorine-18; Bone

Abbreviations: ACP, amorphous calcium phosphate; Co, cortical bone; D-Co, demineralised cortical bone; DPD, 2,3-dicarboxypropane-1, 1-diphosphonate; D-Sp, demineralised spongiosa; EDTMP, ethylenediamine-*N*,*N*,*N*′,*N*′-tetrakis (methylene phosphonic acid); HA, hydroxyapatite; HBSS, Hank's balanced salt solution; MDP, methylene diphosphonate; MRI, magnetic resonance imaging; PP, polyphosphonates; Sp, spongiosa.

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Introduction

Some primary tumours, like those of breast, lung and prostate, have the tendency of metastasising into osseous tissue and therefore the detection of these bone metastases plays an important role in medical imaging techniques. One detection method is bone scanning with radiolabelled polyphosphonates (PP), first described in the early 1970s [1,2]. A prerequisite for the uptake of these PP is good affinity to the bone and even higher affinity to the matrix which is associated with metastatic growth [3,4]. There are controversies about the mechanisms involved in this uptake, ranging from adsorption onto the mineral phase with disputed binding to the organic phase (collagen), over incorporation into the mineralisation process to a combination of both mechanisms. Other factors, such as solubility of the complex, concentration of ligand or effects of the radionuclide, have also been discussed as possible parameters influencing bone uptake [3–9]. Even the preparation method plays an important role for the resulting complex structure consequently influencing bone uptake [10]. Francis et al. [3,4] presented the theory that bone uptake is essentially associated with amorphous calcium phosphate (ACP), which is initially sequestered by the osteoblasts and then follows the crystallisation process towards crystallised hydroxyapatite (HA). In previous studies, the authors followed this hypothesis and investigated a setup of various differently radiolabelled PP in vivo and pre vivo on various artificially manufactured bone compartments such as HA and ACP [10–14]. We found evidence for this ACP and HA hypothesis, since there was agreement between some of our in vitro findings and in vivo results from literature [14]. However, we had to conclude that the behaviour of bone seekers is embossed by a variety of parameters in vivo. This in vivo situation is hardly reproducible by any model. Thus, the next logical step towards a model that reflects the in vivo conditions more authentically was the advancement of the method using human bone tissue instead of synthetic compounds as binding matrix. The use of lyophilised bone allografts is an established technique in orthopaedic surgery. During the preparation of these allografts, the integrity of the important mineral layers is maintained [15]. Following Francis' [3,4] theory of the mineral phase being the crucial factor in bone uptake of PP, we hypothesise that these allografts are an adequate matrix for our further experiments. Hence, the aim of the present study was (1) the preparation of human bone powder from cortical bone (Co) and spongiosa (Sp) in a comparable particle size range to that of HA and ACP, (2) the evaluation of adsorption parameters of the differently radiolabelled PP and [^{18}F]-fluoride and (3) the survey of the findings in comparison with the previously presented model data [14].

Methods

Preparation of allografts

Both cortical allografts and spongiosa chips were prepared according to [15]. Briefly, bone was cut using a low-speed diamond saw (Isomet; Buehler Ltd., Lake Bluff, IL). Lipids were extracted by diethylether renewed 3 times for at least 6 h. The specimens were then rinsed with 70% ethanol for 3 h, 50% ethanol for 3 h and 30% ethanol for 4 h. Bones were then washed with distilled water for 2 h and kept in a solution of 3% hydrogen peroxide for 2 h. The samples were freeze-dried for 5 consecutive days. Residual moisture was less than 5% of final dry weight. Lyophilised specimens were packed under argon gas in heat-sealed plastic bags. Finally, the bone specimens were sterilised with 25 kGy of cobalt irradiation.

Preparation of cortical bone and spongiosa powder

After removing the remaining spongy part, the cortical allografts were crushed to small chips and cooled with liquid nitrogen before grounding in a mill progressively equipped with ring sieves of 4 mm, 0.5 mm and 0.08 mm mesh size. The process yielded 93.8% cortical bone powder with diameter characteristics of 90% < 35.01 μm and 50% < 17.58 μm.

The spongiosa chips were directly pulverised in a mill progressively equipped with ring sieves of 0.5 mm and 0.08 mm mesh size. The process yielded 82.4% powder with diameter characteristics of 90% < 37.01 μm and 50% < 18.46 μm.

Decalcification process and preparation of demineralised cortical bone (D-Co) and spongiosa powder (D-Sp)

Lyophilised cortical bone pieces (1 × 1 × 0.3 cm) and spongiosa chips were weighed and placed in a surplus of 10% HCl. The endpoint of the decalcification process was determined by piercing the material with a needle. Decalcified gummy material did not offer resistance.

Afterwards, the specimens were washed neutral (pH 5–6), freeze-dried and weighed. The mineral phase was calculated to represent 98.43% (±0.69%) of the cortical pieces and 95.47% (±1.55%) of the spongiosa chips.

Freeze-dried samples were pulverised in a mill progressively equipped with ring sieves of 0.5 mm and 0.08 mm mesh size. The process yielded 65%, and the residual moisture was determined to be less than 5%. Particle size analysis yielded diameter characteristics of 90% < 38.18 μm and 50% < 19.51 μm for D-Co or 90% < 38.59 μm and 50% < 19.20 μm for D-Sp, respectively.

Binding experiments on cortical bone and spongiosa

To a vial containing 3 mg of bone powder, 3 ml of Hank's Balanced Salt Solution (HBSS) were added and the vial was swayed at 37°C for 24 h. 0.3 μmol of a radioactive-

labelled PP or 25 MBq [^{18}F]-fluoride was added, the tube was replaced in the water bath (10–120 min, 37°C) and vortexed every 15 min and before extraction.

Binding measurement

An aliquot of 50 μl of this suspension was added to 2 ml of phys. saline. Out of this dilution, 3 aliquots of 50 μl (V_{1-3}) were taken and placed in tubes for the gamma-counter. The rest of the dilution was filtered through a Millex-FG® single use filter unit and 3 aliquots of 50 μl were taken from the filtrate and placed in tubes (N_{1-3}). The radioactivity of the 6 tubes was measured in the gamma-counter and the percentage of irreversibly bound radiolabelled polyphosphonate was calculated as percent binding = 100 * {AM(V_i) − AM(N_i)}/AM(V_i), (AM = arithmetic mean).

Filter experiments

These experiments were intended to correct the data obtained from the binding experiments for activity left on the millipore filters. The procedure was similar to the chapters *binding experiments and measurement*. The only modification was the omission of binding matrix and associated incubation periods.

Filter correction

The blank values obtained from filter experiments were converted via the term: $[FV_M + (100 - V_M)]/100 * FV_M = FV_{1-4}$. After 4 iterative arithmetic operations, FV_4 was obtained and subtracted from V_M ($\geq V_{Fc}$).

FV_M	Filter value, measured in filter experiment
FV_{1-4}	Filter values, calculated iteratively
V_M	Value, measured in binding experiment
V_{FC}	Value, filter corrected

N.c.a.(no carrier added) [^{99m}Tc]-EDTMP

[^{99m}Tc]-TcO_4^- (<600 MBq/5 ml) was added to the Multibone® kit and maintained 30 min at ambient temperature.

[^{99m}Tc]-EDTMP with rhenium carrier

To the [^{99m}Tc]-TcO_4^- (505–890 MBq/5 ml) solution, 11–150 μl (64–870 μmol) perrhenate was added. After addition of 3.5 mg tin(II)chloride, the Multibone® kit was incubated at ambient temperature for 30 min.

[^{99m}Tc]-EDTMP with indium carrier

To the [^{99m}Tc]-TcO_4^- (<400 MBq/5 ml) solution, 2.21 mg indium(III)chloride was added (2 mM). The resulting mixture was transferred into the Multibone® kit and kept 30 min at ambient temperature.

[^{99m}Tc]-EDTMP boiled

Radiolabelled exactly as described in the chapters *n.c.a. [^{188}Re]-EDTMP and [^{188}Re]-EDTMP with rhenium carrier* (vide infra), with [^{99m}Tc]-TcO_4^- (n.c.a.: 1591 MBq; rhenium carrier added: 546.3 MBq) instead of [^{188}Re]-ReO_4^-.

N.c.a.[^{111}In]-EDTMP

[^{111}In]-Indium(III)chloride (< 70 MBq) was diluted with 5 ml phys. saline, added to the Multibone® kit and kept at ambient temperature for 30 min.

[^{111}In]-EDTMP with rhenium carrier

15 μl (87 μmol) perrhenate was added to [^{111}In]-indium(III)chloride (< 10 MBq) in 5 ml phys. saline. 3.5 mg tin(II)chloride was added to the Multibone® kit and incubated with the [^{111}In]-indium(III)chloride/perrhenic acid solution at ambient temperature for 30 min.

[^{111}In]-EDTMP with indium carrier

2.21 mg indium(III)chloride was added to [^{111}In]-indium(III)chloride (< 15 MBq) in 5 ml phys. saline (2 mM). This solution was added to the Multibone® kit and kept at ambient temperature for 30 min.

N.c.a.[^{188}Re]-EDTMP

70 μl of hydrochloric acid (32%) was added to the [^{188}Re]-ReO_4^- solution (5 ml, 83.4 MBq). This mixture was added to the combined contents of two Multibone® kits and kept at 100°C for 60 min. After pH adjustment, the mixture was sterile filtered (5 ml, 23.7 MBq).

[^{188}Re]-EDTMP with rhenium carrier

Solutions of 20 mg stannous chloride dihydrate in 0.3 ml 1 M hydrochloric acid and 30 mg ascorbic acid and 30 mg oxalic acid in 0.6 ml 0.1 M acetate buffer pH 4, respectively, were prepared. Perrhenic acid (11 μl, 64 μmol) was diluted with 0.1 ml 1 M hydrochloric acid. 5 ml of the [^{188}Re]-perrhenate solution (62.3 MBq) was added to the combined contents of two Multibone® kits. The solutions of perrhenic acid, stannous chloride and ascorbic acid/oxalic acid were added, the solution stirred at 100°C for 60 min. The reaction mixture was sterile filtered and neutralised with sterile 0.5 M sodium phosphate buffer (5 ml, 33.2 MBq).

[^{99m}Tc]-DPD and [^{99m}Tc]-MDP

Labelled according to the package leaflet (5 ml) and an aliquot was withdrawn to yield the demanded ligand concentration of 0.1 mM in the incubation suspension.

[^{153}Sm]-EDTMP (Quadramet®) was obtained commercially and diluted to yield the demanded ligand concentration of 0.1 mM in the incubation suspension.

Quality control

Performed for each production with ITLC or HPLC [11] and radiochemical purity exceeded 97%.

Binding reversibility studies

After proceeding a binding experiment with preparations of [^{99m}Tc]-/Re-EDTMP (11 μl and 80 μl perrhenic acid) or [^{18}F]-fluoride following the defined procedure (vide supra), 2 aliquots were taken from the remaining suspension and diluted with HBSS at the ratios 1 + and 1 + 4, respectively. The diluted samples were replaced in the water bath for another 120 min. The workup procedure was done according to *binding measurement*.

Ligand concentration experiments

[^{153}Sm]-EDTMP (Quadramet®) was prepared to yield a ligand concentration of 1 mM EDTMP.

Statistics

Statistical analysis was 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), while those between more groups were made by analysis of variance (ANOVA). A value of $P < 0.01$ was considered significant. Descriptive statistical analysis was performed using mean values and the standard deviation.

General

Multibone® and unformulated EDTMP were commercially obtained (Izotop, Budapest, HUN), [^{153}Sm]-EDTMP (Quadramet®) and DPD (Teceos®) were obtained from Schering AG (Berlin, GER), MDP was obtained from Rotop (Radeberg, GER). Sterile phys. (0.154 M) saline was obtained from Fresenius Kabi (Graz, AUT). Perrhenic acid (70% aqueous), indium chloride (98%), HBSS (H 8264) and HA (21223) were obtained from Sigma-Aldrich Chemical Company (Steinheim, GER). [^{111}In]-$InCl_3$ was obtained from ARC Seibersdorf (Seibersdorf, AUT). ACP (CAS Nr 7758-87-4) was obtained from Applichem (Darmstadt, GER). The Elutec™ [^{99}Mo/^{99m}Tc]-generator was obtained from Bristol-Myers Squibb (Brussels, BEL). The [^{188}W/^{188}Re] generator was obtained from the Oak Ridge National Laboratory (Oak Ridge, TN, USA). Millex FG® 0.22-μm sterile filters were obtained from Millipore® (Bedford, MA, USA). Gamma counting was performed on a Cobra II auto-gamma-counter (Canberra Packard, CAN). [^{18}F]-fluoride was produced via the ^{18}O(p,n)^{18}F reaction in a GE PETtrace cyclotron (16.5 MeV protons) on >97% enriched [^{18}O]-water (Rotem, Leipzig, GER). Thermostatic water bath was a Schüttler 1083 from GFL® (Burgwedel, GER). Dose calibrator was a Curiementor 2 from PTW (Freiburg, GER). A Retsch ZM100 centrifugal mill (Retsch technologies, Haan, GER) equipped with a 12 teeth rotor was used for the grounding processes. Residual moisture was measured with an SMO 01 analyser (Scaltec Instruments, GER). Particle size analyses were performed using a SALD-1100 (Shimadzu, JPN).

Results

Binding kinetics

Data are presented in Fig. 1. A distinct time dependency is visible. Descriptively, binding on Co is higher than binding on Sp throughout the whole kinetic experiment.

Binding experiments on demineralised bone

Data are presented in Table 1 and Fig. 2. The relatively high [^{18}F]-fluoride values on D-Co and D-Sp presented in Fig. 2 are the values obtained after 120 min of incubation (before the reversibility experiment). The comparison between untreated and demineralised matrices revealed a significant decrease in binding on the demineralised form in all cases ($P < 0.01$).

Isotopic effects, influence of preparation, different carrier and ligand concentrations

Data are presented in Table 1 and Figs. 3 and 5. It has been shown earlier [14] that the dilution of the 50 μl suspension-aliquot with 2 ml phys. saline represents a washing process able to remove unbound PP. Data resulting from the ligand concentration experiment are presented in Fig. 3. It is clearly visible that uptake is negatively influenced by increasing amounts of ligand. An increase in ligand concentration resulted in a significant decrease in binding of [^{153}Sm]-EDTMP both on Co and Sp ($P < 0.01$).

Binding reversibility studies

Data are presented in Fig. 4. Irreversibility is evident due to the remaining high binding values after dilution 1 + 1 and 1 + 4 except for [^{18}F]-fluoride on demineralised matrices. ANOVA analysis of the reversibility studies showed no significant differences in binding for undiluted,

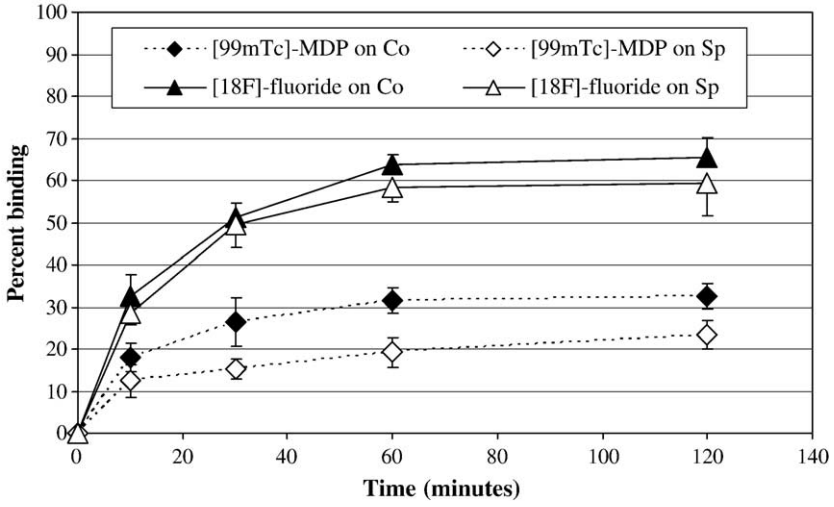

Fig. 1. Binding kinetics of [^{99m}Tc]-MDP and [^{18}F]-fluoride on 3 mg of Co and Sp. Each value represents the filter-corrected arithmetic mean of at least 5 experiments, each measurement performed in triplicate. Error bars represent the standard deviation.

1 + 1 and 1 + 4 experiments except for [^{18}F]-fluoride on demineralised matrices ($P < 0.01$).

Discussion

The role of conventional [^{99m}Tc]-methylene diphosphonate scintigraphy as standard of reference for detection of skeletal metastases from solid tumours or primary bone tumours [16] has recently been challenged by screening tools with similar to higher sensitivity such as whole-body magnetic resonance imaging (MRI) [16–19] or higher sensitivity such as FDG PET [20] or combinations of these imaging techniques [20,21]. Whereas some authors have proposed whole-body MRI as an alternative to skeletal scintigraphy [19], others have argued in favour of a complementary role of whole-body MRI and skeletal scintigraphy [20,22,23]. For example, Daldrup-Link et al. [20] have shown that as an alternative to FDG PET, considering costs and its limited availability, combined whole-body MR imaging and skeletal scintigraphy are less expensive but lead to a comparable sensitivity.

Testing and introduction of new bone seekers with improved binding characteristics might help to clarify the role of skeletal scintigraphy in detecting bone tumours.

Although the exact mechanisms of bone uptake are discussed controversially amongst the community, the involvement of the inorganic bone compartment in this process seems to be undisputed. In autoradiographic analyses of foetal calvarias, Kanishi et al. found high uptake in the area of the osteoblast-like cells whereas no uptake was found in osteoblast-like cells in vitro [6]. Francis et al. described the involvement of ACP structures in the higher uptake of pathologically derailed ossification [3,4]. Clinically, areas of increased osteogenic activity secondary to disease or trauma exhibit higher concentrations of ACP than normal bone [3]. This fact could explain higher uptake in these regions. In contrast, it is well known that areas of osteoclastic activity show reduced uptake. Huigen et al. evaluated adsorption parameters of various PP on ACP finding also pH, composition of the incubation liquid, preparation parameters of the radiotracers and amount of adsorbent influencing the uptake [24]. Adopting Huigen's setup, we recently evaluated a

Table 1
The binding of the evaluated tracers on cortical bone (Co) and spongiosa (Sp) in percent

Tracer	Condition	Co		Sp	
		Mean	±SD	Mean	±SD
[^{99m}Tc]-MDP	120 min	31.65	4.32	20.01	5.30
[^{99m}Tc]-MDP*	120 min	6.20*	1.11	3.77*	0.43
[^{99m}Tc]-DPD	120 min	51.11	5.35	48.27	3.60
[^{99m}Tc]-DPD*	120 min	4.47*	1.11	0.68*	0.21
[^{153}Sm]-EDTMP	120 min	27.79	2.83	26.7	1.22
[^{153}Sm]-EDTMP*	120 min	2.12*	1.27	1.87*	1.38
[^{18}F]-fluoride	120 min	65.29	4.73	59.19	7.76
[^{18}F]-fluoride*	120 min	28.68*	0.93	24.58*	3.14
[^{99m}Tc]-EDTMP	120 min	5.65	1.32	4.24	1.51
[^{99m}Tc]-EDTMP boiled	120 min	8.18	1.58	7.68	2.25
[^{99m}Tc]-/Re-EDTMP 11 µl	120 min	5.94	2.04	4.54	1.18
[^{99m}Tc]-/Re-EDTMP 11 µl boiled	120 min	23.65	3.20	15.03	1.97
[^{99m}Tc]-/Re-EDTMP 15 µl	120 min	7.78	4.59	5.93	2.20
[^{99m}Tc]-/Re-EDTMP 80 µl	120 min	13.02	2.79	13.02	1.41
[^{99m}Tc]-/Re-EDTMP 150 µl	120 min	12.20	3.85	12.40	2.84
[^{99m}Tc]-/In-EDTMP	120 min	7.54	0.86	6.58	1.31
[^{188}Re]-EDTMP	120 min	7.73	2.32	2.23	2.66
[^{188}Re]-/Re-EDTMP	120 min	3.84	1.65	2.02	1.41
[^{111}In]-EDTMP	120 min	11.43	1.76	8.68	2.75
[^{111}In]-/In-EDTMP	120 min	13.20	4.47	7.89	2.27
[^{111}In]-/Re-EDTMP	120 min	17.18	1.66	14.50	1.85

Each value represents the filter-corrected arithmetic mean of at least 5 experiments, each measurement performed in triplicate (3 mg matrix, mean ± standard deviation).
Data marked with an asterisk (*) represent binding on demineralised matrices (i.e., D-Co and D-Sp, respectively).

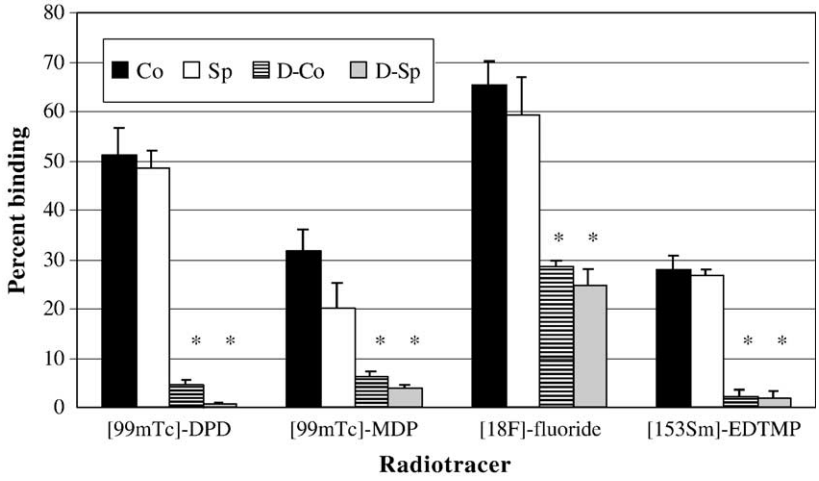

Fig. 2. Binding of selected tracers on demineralised bone matrix in comparison to untreated matrix. Each value represents the filter-corrected arithmetic mean of at least 5 experiments (3 mg matrix, 120 min), each measurement performed in triplicate. Error bars represent the standard deviation. All values result from undiluted experiments. Significant differences ($P < 0.01$) are marked with an asterisk.

series of PP and [^{18}F]-fluoride on ACP, HA and bovine collagen [14] and found negligible uptake in the organic fibres. In that former study, we hypothesised – in agreement with the above mentioned studies – that uptake is mainly restricted to the mineral phase of bone. The whole study was conducted with 3 mg of the respective adsorbent and this procedure was adopted for the present follow-up examination.

Adsorption kinetics

As already presented with [^{99m}Tc]-MDP and [^{18}F]-fluoride on ACP and HA [14], a distinct time dependency of binding was observed also on Co and Sp (cf. Fig. 1). The adsorption kinetics on Sp and Co are slower than those found on HA and ACP [14]. Descriptively, cortical uptake was higher than spongiosa uptake throughout the whole kinetic experiment.

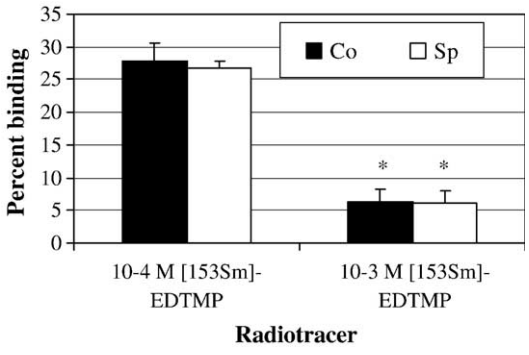

Fig. 3. Binding rates of [^{153}Sm]-EDTMP at two different ligand concentrations (10^{-4} and 10^{-3} M) on Co and Sp (3 mg, 120 min). Each value represents the filter-corrected arithmetic mean of at least 5 experiments, each measurement performed in triplicate. Error bars represent the standard deviation. Significant differences ($P < 0.01$) are marked with an asterisk.

Binding studies on demineralised bone

This experiment was designed in order to verify whether uptake was restricted exclusively to the mineral compartment or whether – after demineralisation – significant uptake was detectable also on the remaining organic compartment. Schwartz et al. [25] and Sela et al. [26] hypothesised that the organic phase is strongly involved in the uptake process of [^{99m}Tc]-MD[^{32}P]. This was explained by the dissociation of the [^{99m}Tc] and MD[^{32}P] moieties, with the former predominantly accumulating in the organic phase and the latter in the mineral phase. Fig. 2 shows very low binding rates on this organic matrix compared to the untreated bone powders, although the same amount of matrix (3 mg) was provided for binding. Again, binding on the cortical fractions was higher than on spongiosa. Interestingly, the binding of [^{18}F]-fluoride on demineralised bone was initially high but the reversibility studies exhibited reversible binding (cf. Fig. 4). Therefore, since (1) uptake on this organic tissue was low and (2) highly reversible and (3) the demineralisation process afforded very little organic residue (<5%), a significant contribution of this organic compartment to the overall bone uptake of radiotracers appears to be unlikely.

Isotopic effects, influence of preparation, different carrier and ligand concentrations

Fig. 5 presents preparations of EDTMP radiolabelled with different radionuclides and carriers (see also Table 1). Descriptively, [^{111}In] increases the uptake of EDTMP in each case compared to similarly prepared [^{99m}Tc]-analogues: [^{111}In]-EDTMP > [^{99m}Tc]-EDTMP, [^{111}In]-/In-EDTMP > [^{99m}Tc]-/In-EDTMP and [^{111}In]-/Re-EDTMP (15 μl) > [^{99m}Tc]-/Re-EDTMP (15 μl). Remarkable is the fact that, as already presented recently [14], simple modifications of a commercially available EDTMP kit can increase the average

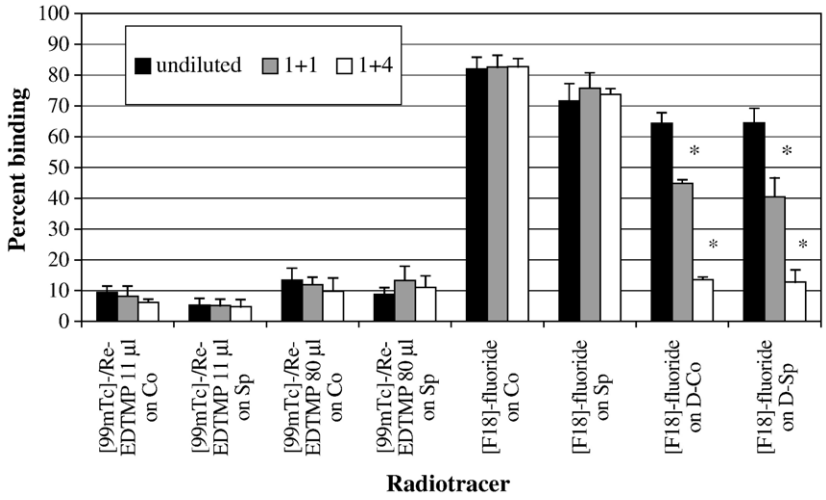

Fig. 4. Binding reversibility of various bone seekers (3 mg, 240 min). Each bar represents the arithmetic mean value of at least 5 experiments, each measurement performed in triplicate. Error bars represent the standard deviation. Significant differences ($P < 0.01$) are marked with an asterisk.

uptake manifold. These findings were interpreted by the rearrangement of complex structure and/or formation of polymeric structures after addition of carrier or boiling. [^{188}Re]-EDTMP shows higher binding than the carrier added analogue, which contravenes the recently published in vivo findings of [^{188}Re]-PP [27–29]. However, these findings are consistent on both artificial bone material and human material. Table 1 clearly shows the influence of the amount of Re-carrier on the binding of [^{99m}Tc]-EDTMP. Remarkable is also the unrivalled high uptake of [^{18}F]-fluoride, showing no reduced uptake on Co and Sp as compared to HA and ACP. These findings could be explained by the fact that [^{18}F]-fluoride in contrast to the ligand-based radiotracers exhibits negligible amount of substance. Co and Sp naturally present

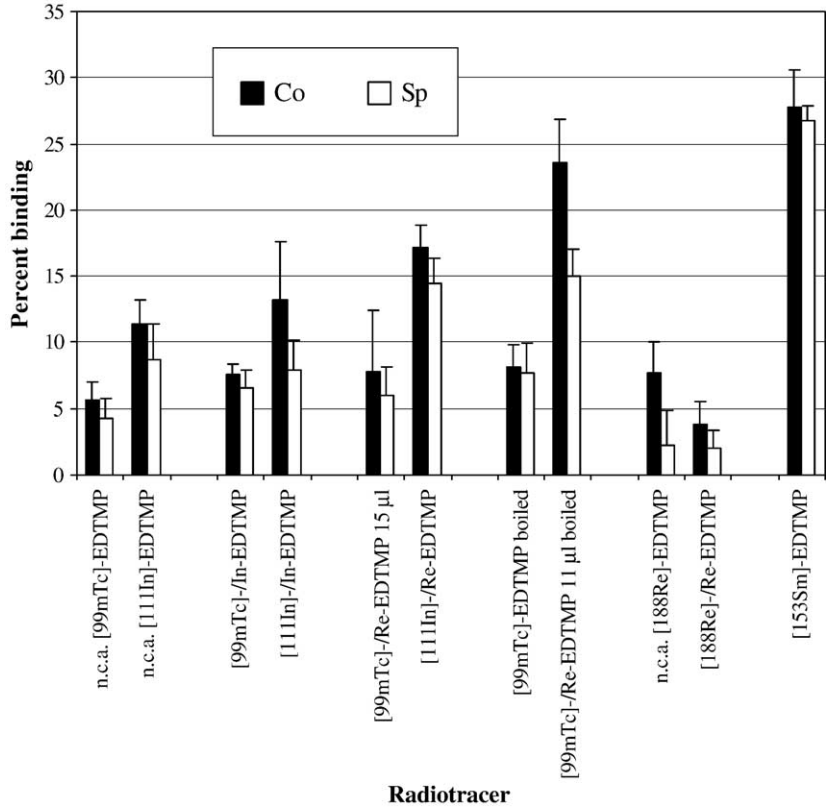

Fig. 5. Binding of differently radiolabelled EDTMP with different carriers on Co and Sp (3 mg, 120 min). Each value represents the filter-corrected arithmetic mean of at least 5 experiments, each measurement performed in triplicate. Error bars represent the standard deviation.

lower amounts of binding sites for adsorption than pure binding material as used in [14], being the limiting factor for ligand-based tracers but not for picomolar [^{18}F]-fluoride. The influence of ligand concentration is presented in Fig. 3 by dint of [^{153}Sm]-EDTMP. These findings are in a row with the dilution effects already found for ACP and HA [14]. This fact demonstrates the importance of correct and similar preparation of the radioligand for comparative bone uptake studies in vitro and in vivo.

Binding reversibility studies

Binding reversibility was assessed using the same method as presented recently [14]. Fig. 4 clearly evinces that binding reaches a high degree of irreversibility except [^{18}F]-fluoride on D-Co and D-Sp.

Do these findings verify the pre vivo model?

Recently, the authors discussed a variety of correlations between uptakes on HA and ACP compared to in vivo findings [14]. Fig. 6 presents a comparison of all so far evaluated matrices, from these recent experiments and the present study. In this figure, uptake rankings of the most prominent tracers on artificial matrix were compared with human matrix. The highest degree of correlation was found in part A, comparing HA and Co. Only [^{18}F]-fluoride and [^{99m}Tc]-MDP are interchanged, probably due to the abovementioned amount of substance effects. The other 3 parts of the figure (B–D) show a higher variability (especially part C), but neither comparison exhibits a higher divergence than three ranks. We also calculated a ratio between the mean of ACP and HA values and the mean of Co and Sp for each evaluated tracer and found an overall ratio of 2.3 (±1.2). This value shows twofold uptake on the artificial compared to the human matrices. Since (1) our decalcification experiment exhibited that more than 95% of the human matrix was mineral phase and (2) uptake on the remaining organic phase uptake was negligible, we hypothesise that these differences in uptake result from differently arranged matrix and not from different amount of mineral phase. Since we present evidence that binding is restricted to the mineral compartment of bone and there is a high degree of correlation between uptakes on human matrix compared to HA, the concept of adsorption studies on artificial matrix does reflect the behaviour of bone seekers on human tissue. However, the behaviour of bone seekers is embossed by a variety of parameters in vivo. This by-far more complex situation in vivo is hardly reproducible by any model. For the evaluation of the interaction between matrix and bone seekers, however, the presented model could be helpful. The model could even be a prerequisite in the development phase of new bone seekers.

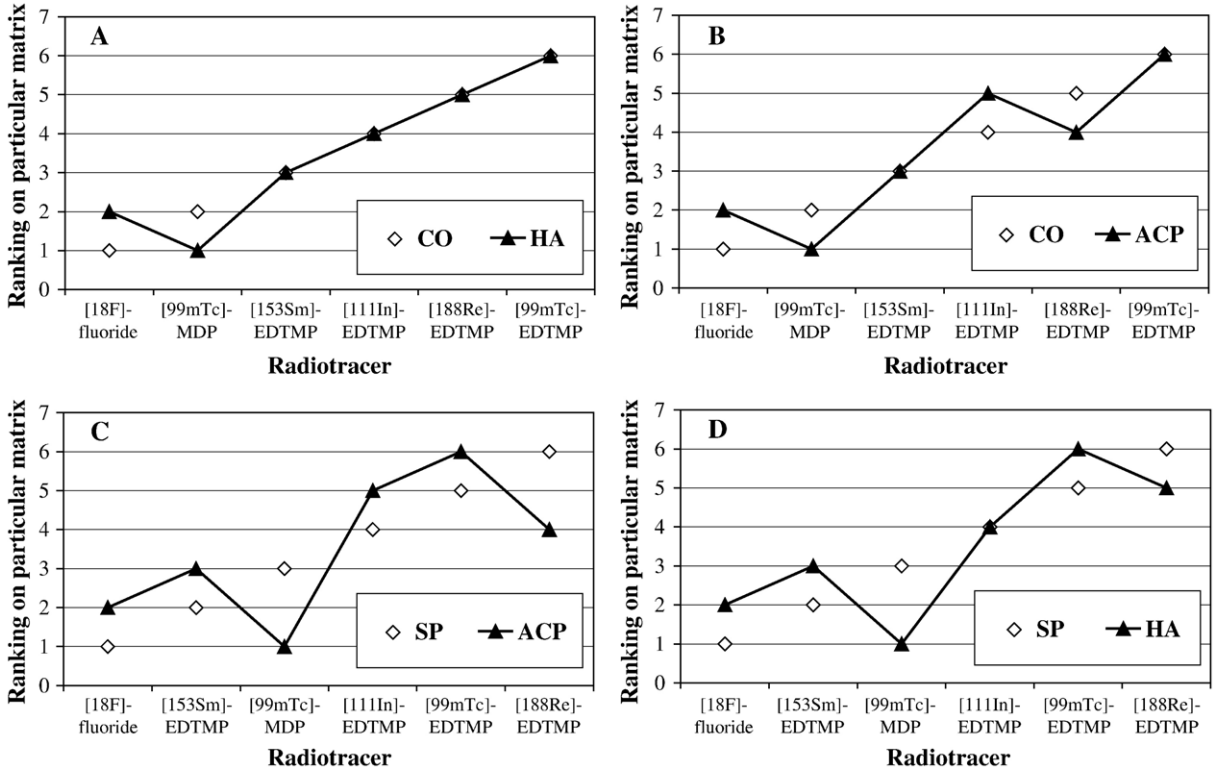

Fig. 6. Comparison of the ranking orders of n.c.a. radiolabelled EDTMP preparations, [^{18}F]-fluoride and [^{99m}Tc]-MDP on (A) Co and HA, (B) Co and ACP, (C) Sp and ACP and (D) Sp and HA (binding values on HA and ACP are taken from [14]). The rankings are calculated on basis of the binding values on 3 mg of the particular matrix at 120 min.

In conclusion, the evaluation of bone tracers on the presented preparations from human bone allografts is reproducible and feasible. The binding of the evaluated bone seekers is mainly restricted to the inorganic phase and on these human bone matrices follow a comparable pattern as recently found on artificial bone preparations (HA and ACP). Thus, artificial bone preparations are equivalent to human bone preparations. The present study substantiates the recent findings that for the evaluation of the interaction between matrix and bone seekers, the recently presented model could be a time-saving and helpful method.

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