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In vitro evaluation of no carrier added, carrier added and cross-complexed [^{90}Y]-EDTMP provides evidence for a novel "foreign carrier theory"

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Abstract

The present study focused on the preparation of novel bone tracers containing yttrium as radionuclide or carrier. Moreover, these preparations were comparatively evaluated in vitro on the basis of a recently presented pre vivo model comprising binding studies on synthetic and human bone powder. It was shown that among the therapeutic radionuclides, no carrier added [^{90}Y]-EDTMP exceeded [^{188}Re]-EDTMP while yielding lower binding values than [^{153}Sm]-EDTMP. Furthermore, the authors investigated the influence of "foreign" carriers added to [^{90}Y]-EDTMP, [^{99m}Tc]-EDTMP and [^{111}In]-EDTMP by the method of cross-complexation. The findings reveal a new paradigm: a carrier more foreign to the complexed radionuclide causes a higher binding increase on human bone matrices in vitro than a more "related" carrier.

Keywords: Bone pain palliation; [^{90}Y]-EDTMP; Cross-complexation; Binding studies; Foreign carrier theory

1. Introduction

Metastatic bone pain is the most common pain syndrome encountered in breast, lung and prostate cancer patients [1]. The often torturous pain associated with bone metastases and the resulting limitation in motility considerably compromises the life quality of the patients. Therefore, alleviation of pain arising from bone metastases is a great challenge for medical science. Systemic radionuclide therapy provides an effective means for bone pain palliation in patients with multifocal bone metastases [2,3]. The optimum characteristics of a radionuclide to be considered as an effective radiopharmaceutical for palliative pain relief include a particulate emission of an appropriate energy and range, a physical half-life that approaches the biological half-life of the radiopharmaceutical in the tumor, selective concentration in bone lesions, rapid blood clearance and low extra-osseous uptake. In all cases, the radionuclide used is a β-emitting isotope with high energy emission and an effective range of only a few millimeters. In order to target bone tissue, the β-emitting nuclide is bound to an inactive compound that transfers the isotope to the lesion and allows a located energy transfer to the metastasis and surrounding bone. Although the exact mechanism of pain relief is not known, it is discussed that the improvements are caused by cytotoxic effects on bone cells, thereby inhibiting the release of pain mediators.

Yttrium-90 (^{90}Y) is produced by neutron activation of yttrium-89 and decays by β-particle emission (E_{max} = 2.28 MeV) with a half-life of 2.67 days. The use of ^{90}Y as a therapeutic radionuclide for bone pain palliation has been

Abbreviations: AV, atomic volume; CA, carrier added; Co, cortical bone; EDTMP, ethylenediamine-*N*,*N*,*N*′,*N*′-tetrakis (methylene phosphonic acid); HA, hydroxyapatite; HBSS, Hank's balanced salt solution; NCA, no carrier added; PP, polyphosphonate; Sp, spongiosa.

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successful for decades [4], and stable complexes formed with citrate or EDTMP have been clinically used [5]. However, due to the fact that ^{90}Y is a pure β emitter, in vivo evaluations like pharmacokinetic studies and calculations of radiation doses to metastases and unaffected organs are impossible to realize. Several approaches to overcome these shortcomings — including the substitution of ^{90}Y for the positron-emitting ^{86}Y isotope — have been published [6–9].

In previous studies, the authors investigated a setup of various bone-seeking agents produced by the novel technique of "cross-complexation" in vitro and in vivo [10–12]. The term cross-complexation describes the addition of a stable nuclide to a radioactive complex preparation. The so-called carrier can either be "related" or "foreign" to the radionuclide, depending on their physical properties like electron configuration, electronegativity according to Pauling and atomic volume (AV). Because the electron configuration is of great importance for the coordination sphere of a complex, Y is closer related to In (both three valence electrons, related) than to Re (3 vs. seven valence electrons; foreign). Furthermore, Tc is very closely related to Re (both seven outer electrons, both in the seventh group of the periodic table of the elements, related) and clearly different to In and Y (foreign). For further distinction, the AV can be considered. Regarding technetium (AV=8.5 cm^3/mol) as reference radionuclide, the following carriers would become more foreign in the order: Re (AV=8.85 cm^3/mol)<In (AV=15.7 cm^3/mol)<Y (AV=19.8 cm^3/mol).

Recently, the authors presented a feasible model for the pre vivo evaluation of bone seekers [13] by performing binding studies of various radiotracer formulations on artificially manufactured bone compartments such as hydroxyapatite (HA) and amorphous calcium phosphate. Furthermore, we were able to fortify the model presenting correlations between some of our in vitro findings and in vivo data from literature [13]. In a follow-up study, the authors could verify the model by substituting the synthetic binding matrices for human bone powder [14].

Based on these previous investigations, the aims of the present study were (1) the production of a set of radiotracers comprising no carrier added (NCA), carrier added (CA) and cross-complexed [^{90}Y]-EDTMP, as well as established bone seekers cross-complexed with a stable yttrium isotope, (2) the evaluation of these formulations on HA and human bone powder and (3) the survey of the binding characteristics of the yttrium-containing preparations in comparison to other already evaluated radiopharmaceuticals.

2. Materials and methods

2.1. General

Multibone and unformulated EDTMP were commercially obtained (Izotop, Budapest, Hungary). Sterile phys. saline (0.154 M) was obtained from Fresenius Kabi (Graz, Austria). Perrhenic acid (70% aqueous), indium chloride (98%), Hank's balanced salt solution (HBSS) (H 8264), HA (21223) and yttrium chloride hexahydrate (>99.9%) were obtained from Sigma-Aldrich (Steinheim, Germany). [^{111}In]-$InCl_3$ was obtained from ARC Seibersdorf (Seibersdorf, Austria). [^{90}Y]-YCl_3 was obtained as Yttracis from Schering (Berlin, Germany). The Elutec [^{99}Mo/^{99m}Tc]-generator was obtained from Bristol-Myers Squibb (Brussels, Belgium). Millex-FG 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, Canada). The thermostatic water bath was a Schüttler 1083 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, JPN).

2.2. Preparation of human cortical bone powder and spongiosa powder

The preparation of these samples followed the methods reported elsewhere [14,15]. Briefly, bone specimens were excised from donors, washed, freeze-dried and sterilized. Afterward, these allografts were processed into a fine powder by milling. Measurements revealed a particle size in a comparable range to commercially available HA.

2.3. Radiotracer preparation

Unless not specified below, the preparations followed the specifications reported previously [14].

[^{90}Y]-EDTMP: [^{90}Y]-Yttrium(III)chloride (<7 MBq) was diluted with 5 ml phys. saline, added to the Multibone kit and kept at ambient temperature for 30 min.

[^{90}Y]-EDTMP with rhenium carrier: 15 μl (87 μmol) perrhenate was added to [^{90}Y]-yttrium(III)chloride (<8 MBq) in 5 ml phys. saline. Tin(II)chloride (3.5 mg) was added to the Multibone kit and incubated with the [^{90}Y]-yttrium(III)-chloride/perrhenic acid solution at ambient temperature for 30 min.

[^{90}Y]-EDTMP with indium or yttrium carrier: 2.21 mg indium(III)chloride or 3 mg yttrium(III)chloride were added to [^{90}Y]-indium(III)chloride (<11 MBq) in 5 ml phys. saline (2 mM). This solution was added to the Multibone kit and kept at ambient temperature for 30 min.

[^{99m}Tc]-EDTMP with yttrium carrier: To the [^{99m}Tc]-TcO_4^- (<400 MBq per 5 ml) solution, 3 mg yttrium(III)-chloride was added (2 mM). The resulting mixture was transferred into the Multibone kit and kept for 30 min at ambient temperature.

[^{111}In]-EDTMP with yttrium carrier: 3 mg yttrium(III)-chloride were 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.

Quality control was performed for each production with ITLC or HPLC [10] and radiochemical purity exceeded 97%.

Table 1
The percent binding of the evaluated tracers (0.3 μmol) on the filter. Each value represents the arithmetic mean of at least five experiments, each measurement performed in triplicate

Tracer	Mean±S.D.
[^{90}Y]-EDTMP	4.21±0.95
[^{90}Y]-/Y-EDTMP	1.53±0.50
[^{90}Y]-/Re-EDTMP	0.60±0.29
[^{90}Y]-/In-EDTMP	1.27±0.59
[^{99m}Tc]-/Y-EDTMP	0.81±0.65
[^{111}In]-/Y-EDTMP	3.72±0.19

2.4. Binding experiments

To a vial containing 3 mg of bone powder or HA, 3 ml of HBSS was added and the vial was swayed at 37°C for 24 h. Radioactive-labeled polyphosphonate (PP) (0.3 μmol) was added; the tube was replaced in the water bath (120 min, 37°C) and vortexed every 15 min and before extraction.

2.5. 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 ($V_{1–3}$) 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 three aliquots of 50 μl were taken from the filtrate and placed in tubes ($N_{1–3}$). The radioactivity of the six tubes was measured in the gamma-counter, and the percentage of irreversibly bound radiolabeled PP was calculated as percent binding=$100\{AM(V_i)-AM(N_i)\}/AM\ (V_i)$ (AM= arithmetic mean).

2.6. Filter experiments

The procedure was similar to the Binding Experiments and Binding Measurement. The only modification was the omission of binding matrix and associated incubation periods.

2.7. Filter correction

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

2.8. 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). A value of $P<.05$ was considered significant. Descriptive statistical analyses were performed using mean values and S.D.s.

3. Results and discussion

Treatment of severe bone pain benefits from the β decay of radionuclides like ^{153}Sm, ^{188}Re or ^{90}Y, which are responsible for the palliative effects in cancer patients. However, pure β emitters like ^{90}Y come along with disadvantages regarding in vivo evaluation of distribution or binding behavior. Therefore, in vitro studies could help to clarify binding characteristics and could allow the comparison of novel preparations pre vivo. For the estimation of these binding characteristics, the authors recently evaluated a method that 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 [13,14].

Prior to the binding assay, filter experiments were performed in order to determine the amount of radioactivity lost by the filtering process. Values shown in Table 1 were

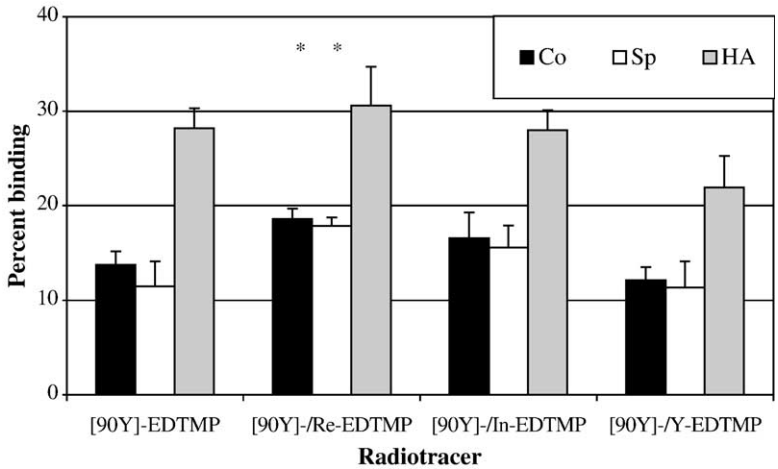

Fig. 1. The binding of NCA and CA [^{90}Y]-EDTMP on HA, human Co and Sp bone powder in percent. Each value represents the filter-corrected arithmetic mean of at least five experiments, each measurement performed in triplicate (3 mg matrix, mean±S.D.). Significant differences from NCA [^{90}Y]-EDTMP values ($P<.05$) are marked with an asterisk.

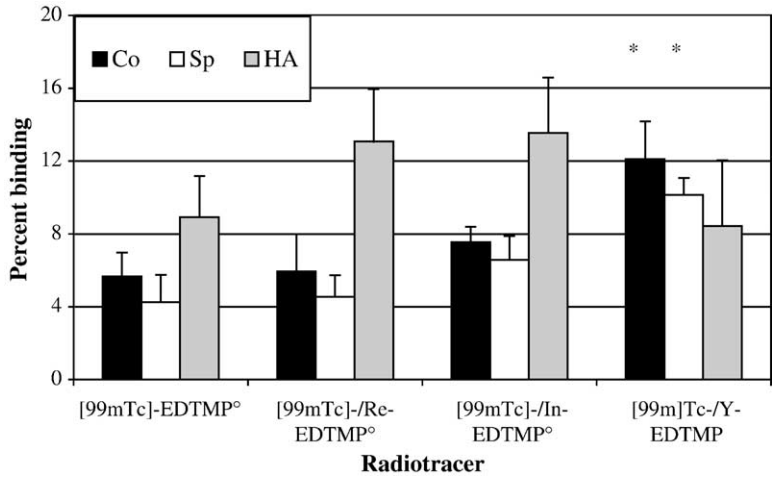

Fig. 2. The effect of different carriers added to [^{99m}Tc]-EDTMP in percent binding. Each value represents the filter-corrected arithmetic mean of at least five experiments, each measurement performed in triplicate (3 mg matrix, mean±S.D.). Significant differences from NCA [^{99m}Tc]-EDTMP, [^{99m}Tc]-/Re-EDTMP and [^{99m}Tc]-/In-EDTMP values ($P<.05$) are marked with an asterisk. Values of marked radiotracers (°) were taken from [13,14].

subtracted iteratively to yield the corrected binding values of the radiotracers presented in Figs. 1–3. Generally, values on HA exceed values on cortical bone (Co) and spongiosa powder (Sp), an effect that was also observed previously [13,14]. This finding may be explained by the influence of different crystal structures that distinguish synthetic from human matrix. Only yttrium carrier added [^{99m}Tc]-EDTMP showed a different binding pattern (Fig. 2).

No carrier added [^{90}Y]-EDTMP yielded 13.7% (±1.44) on Co, 12.2% (±2.79) on Sp and 28.2% (±2.14) on HA (Fig. 1). In comparison to the recently evaluated radiotherapeutics [^{153}Sm]-EDTMP and [^{188}Re]-EDTMP [13,14], binding values of NCA [^{90}Y]-EDTMP were in between. On the one hand, it shows significantly lower uptake than the ^{153}Sm-labeled counterpart that yielded 27.79% (±2.83) on Co, 26.7% (±1.22) on Sp and 52.3% (±1) on HA [13,14]. On the other hand, statistical analyses demonstrated that NCA [^{90}Y]-EDTMP on all matrices significantly exceeds [^{188}Re]-EDTMP, another currently discussed radiopharmaceutical ($P=.002$ on Co, $P=.0001$ on Sp and $P=3.1\times10^{-05}$ on HA).

As shown in Fig. 1, the addition of any carrier to [^{90}Y]-EDTMP influenced the amount of binding on all evaluated matrices. Significant differences were found between NCA [^{90}Y]-EDTMP and [^{90}Y]-/Re-EDTMP on the human matrices ($P=.002$ on Co, $P=.03$ on Sp). The average percent binding value of [^{90}Y]-/Re-EDTMP on HA was insignificantly higher ($P=.46$). In contrast, the addition of indium or yttrium carrier to [^{90}Y]-EDTMP resulted in decreased uptake levels comparable to the NCA formulation. This influence of carrier addition could be interpreted by the rearrangement of complex structures

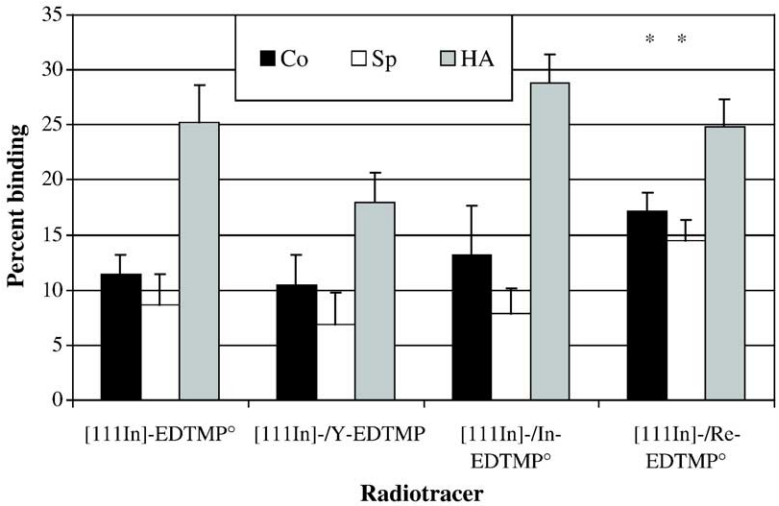

Fig. 3. The effect of different carriers added to [^{111}In]-EDTMP in percent binding. Each value represents the filter-corrected arithmetic mean of at least five experiments, each measurement performed in triplicate (3 mg matrix, mean±S.D.). Significant differences from NCA [^{111}In]-EDTMP values ($P<.05$) are marked with an asterisk. Values of marked radiotracers (°) were taken from Ref. [13,14].

or formation of polymeric structures initiated by the stable nuclides [16]. Interestingly, in the case of [^{90}Y]-EDTMP, foreign carriers like indium and especially rhenium seem to induce higher affinity of the complex to the matrices than the yttrium carrier.

In order to illuminate the role of yttrium as foreign carrier, we investigated the binding behavior of yttrium carrier added [^{99m}Tc]-EDTMP. Fig. 2 demonstrates the positive influence of yttrium carrier addition to [^{99m}Tc]-EDTMP on human bone matrices. Significant differences in the scores were seen between NCA [^{99m}Tc]-EDTMP and [^{99m}Tc]-/Y-EDTMP on Co (P=.003) and Sp (P=.0009), as well as between [^{99m}Tc]-/Y-EDTMP and the indium or rhenium carrier added formulations. Again, the more foreign carriers like indium and especially yttrium caused a higher increase in the uptake behavior than rhenium, which is often used as substitute for technetium regarding structure determination studies [16].

Finally, to further strengthen the "foreign carrier theory," we were interested in the differences between various carrier added [^{111}In]-EDTMP preparations. As depicted in Fig. 3, yttrium and indium carriers had no remarkable influence on the uptake, whereas rhenium was the only carrier that caused significant differences compared to the NCA counterpart ($P=3\times10^{-05}$ on Co, P=.0004 on Sp). Regarding indium, rhenium may be considered more foreign than yttrium. Yttrium, in contrast, is more similar to indium, which is demonstrated by the fact that ^{111}In is sometimes clinically used as a chemical and biological surrogate for ^{90}Y-labeled therapeutic agents [17].

4. Conclusion

The successful preparation of all the presented carrier added formulations allowed insights into a new aspect of understanding CA and cross-complexed radiotracer preparations. Evidence is provided for the novel theory 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. However, this paradigm should be validated in a follow-up in vivo study with tracers providing feasible radiation properties. In the case of radiotracers comprising pure β-emitting nuclides like ^{90}Y, the presented pre vivo method seems to provide a practicable, and so far the only, alternative to estimate their binding characteristics without substitution of the radionuclide.

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