Phosphonate complexes of Gallium-68 for bone tumor imaging

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Introduction: As ^{99m}Tc-phosphonates are well established tracers for the diagnoses of bone metastases using SPECT, analogue attempts for PET using the Germanium-68/Gallium-68 generator based ^{68}Ga tracers would be potentially useful. Therefore molecules / ligands containing phosphonate structures with binding affinities to hydroxyapatite and being adequate complexing agents for trivalent Gallium could be considered as interesting targeting vectors for the synthesis of generator-based PET-tracers for skeletal imaging. The aim of the study was to synthesize complexes with different types of phosphonate ligands in order to understand the rational of ^{68}Ga-phosphonates related to apatite binding.

Experimental: Germanium-68 (T½ = 270.95 d) provides the positron emitter Gallium-68 (T½ = 67.7 min) as an easily available and relatively inexpensive source of a PET nuclide for labeling interesting targeting vectors. Germanium-68 is fixed on a solid phase of titanium dioxide. Through HCl Gallium-68 is eluted from the generator and immobilized on an acidic cation exchanger. Impurities such as zinc, iron and titanium as well as ^{68}Ge generator breakthrough are removed by a special mixture of acetone and hydrochloric acid (N1). Subsequently, ^{68}Ga is eluted in 400 µL of a second mixture of acetone and HCl (N2) from the cation exchanger.[1]
As proof-of-concept, the phosphonate ligands EDTMP (Ethylenediaminetetra(methylene phosphonic acid)), DOTP (1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetra(methylene phosphonic acid)), and DO3AP-ABn (1,4,7,10-Tetraazacyclododecane-4,7,10-triacetic-1-{methyl[(4-aminophenyl)methyl)]phosphinic acid}), have been selected. Beside these the following ligands NOTA, NO2AP, NOA2P, NOTP and DOTA were also tested. Compounds NOTA, NO2AP, NOA2P, NOTP and DOTP have been obtained from C. F. Geraldes, DO3AP-ABn from I. Lukeš and EDTMP from Sigma-Aldrich.

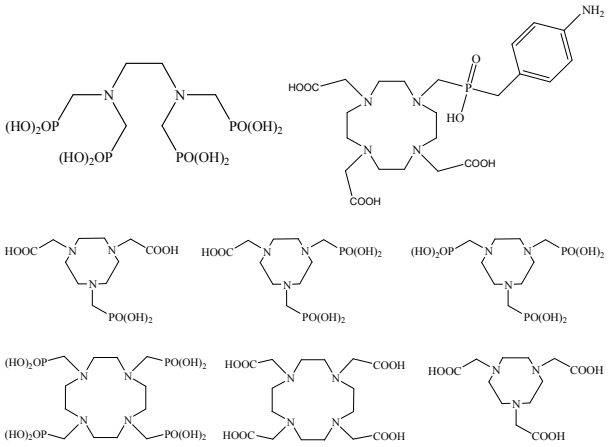

Fig. 1: used ligands EDTMP, DO3AP-ABn, NO2AP, NOA2P, NOTP, DOTP, DOTA, NOTA (top left to bottom right)

^{68}Ga labeling is performed in 400 µL 0.12 M Na-HEPES buffer by adding the ^{68}Ga fraction of N2. Through variation of reaction time, temperature, pH and different amounts of the ligands, optimum reaction parameters for complex formation were tested. Analyses of radiochemical yield are carried out by TLC on cellulose using two liquids (A: water:ethanol:pyridine 4:2:1; B: isotonic saline). Binding studies on synthetic hydroxyl apatite Hap were carried out to simulate the binding of the different complexes ^{68}Ga-phosphonates to bone structures.

Results and Discussion: The elution of ^{68}Ga from the generator and the on line-processing of the eluate are performed within five minutes only. Labeling proceeds at temperatures between 25-60°C within 2-10 min in a total volume of 800 µL. Ligands are used in nanomole amounts only and the radiochemical yields are 50 to 95%. The most promising complex concerning synthesis is ^{68}Ga-EDTMP with a radiochemical yield of 95% in 5 min. More precise results were presented in literature.[2]
Hydroxyapatite binding assays show strong and fast binding of ^{68}Ga-EDTMP and ^{68}Ga-DOTP (> 90% within 10 min), while ^{68}Ga-DO3AP-ABn is not binding. As proof of concept, none-phosphonate ligands were tested too and showed the expected results of no binding. The triazacyclononane phosphonate derivatives show an ascending binding to HAp with increasing number of phosphonate groups (8%, 12% and 55% for NO2AP, NOA2P and NOTP) but still much lower than ^{68}Ga-EDTMP or DOTP.
^{68}Ga-EDTMP and ^{68}Ga-DOTP were tested in vivo with good results for ^{68}Ga-EDTMP. Due to low radiochemical yield, the ^{68}Ga-DOTP PET image obtained showed uptake on bone but with worth statistics.

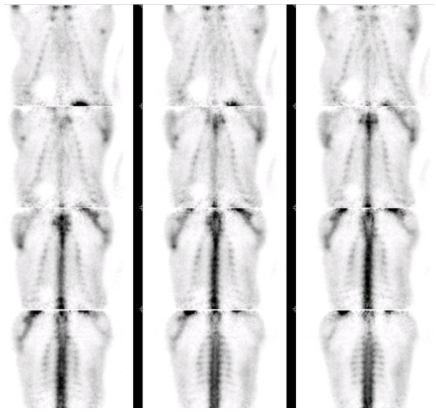

Fig. 2: Bone uptake ^{68}Ga-EDTMP, 1.7 mg ligand, 73 MBq injected into the tail veine of a wistar rat, 0.7 mL injected volume, coronal slices for 30-60 min p.i,

Conclusion: Syntheses of ^{68}Ga complexes are performed within 20 minutes after elution of the generator. First evaluations on synthetic apatite show high binding in a short time for both ^{68}Ga-EDTMP and the macrocyclic ^{68}Ga-DOTP. µ-PET imaging on wistar-rats demonstrated bone uptake *in vivo* for ^{68}Ga-EDTMP and ^{68}Ga-DOTP.
Interestingly, phosphonate functionalities at chelate ligands are not a guarantee for fast and high binding to HAp. Probably at least one free phosphonate group is required.

References

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[2] M. Fellner, P. Riß, N. Loktionova, K. Zhernosekov, F. Rösch, ex vivo-Untersuchung phosphonathaltiger ^{68}Ga-Komplexe an Apatit, Jahresberichte 2006 – Institut für Kernchemie