Synthesis and preliminary evaluation of novel ^{68}Ga-DO2A-tyrosine derivatives

C. Burchardt[1], P. Riss[1], O. Prante[2], O. Thews[3], F. Rösch[1]

[1]Institute of Nuclear Chemistry, Johannes-Gutenberg-University Mainz
[2]Clinic of Nuclear Medicine, Friedrich-Alexander-University Erlangen
[3]Institute of Physiology and Pathophysiology, Johannes-Gutenberg-University Mainz

Introduction: Some radiolabelled amino acids like [^{11}C]MET, 2-[^{18}F]Tyrosine or 2-O-[^{18}F]Fluoroethyltyrosine ([^{18}F]FET) are already established as tumour tracer for Positron Emission Tomography (PET). ^{68}Ge/^{68}Ga-generator produced ^{68}Ga is an upcoming PET nuclide because of its availability and comparably low costs. Consequently, the next challenge is to combine the advantages of labelled amino acids and ^{68}Ga as radionuclide to create a ^{68}Ga-labelled amino acid for PET/CT tumour imaging. We chose DO2A(DO3A) as chelator for ^{68}Ga(III) and tyrosine as amino acid targeting vector. DO2A has the advantage that it allows to couple two amino acids to one chelator, which may increase the capacity of the targeting vector. These compounds should show a considerably increased tumour affinity with a sufficient stability for a PET-measurement in comparison to a one-amino-acid-to-one-chelator compound. In addition, we used different alkyl- and PEG-spacer to vary the distance between chelator and amino acid [1].

Experimental: DO2A was synthesized after a modified method of Kovacs and Sherry starting from cyclen [2]. Different 1-bromo-n-nhloro compounds were used as spacer. These were coupled in a nucleophilic substitution to the phenolic hydroxyl group of the N-tBu and O-Me-protected tyrosine. After a Finkelstein exchange the chelator and the amino acid were combined in another nucleophilic substitution. The protective groups were cleaved with 1 M NaOH/dioxane and TFA. The following four substances and their DO3A-analoges as references were synthesized:

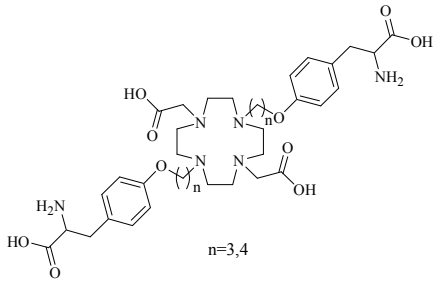

Fig.1: DO2A-tyrosine drivatives with alkyl spacer, the cell and PET studies were carried out with n=4, DO2A-Butyl-Tyrosin (DO2A-Bu-Tyr)

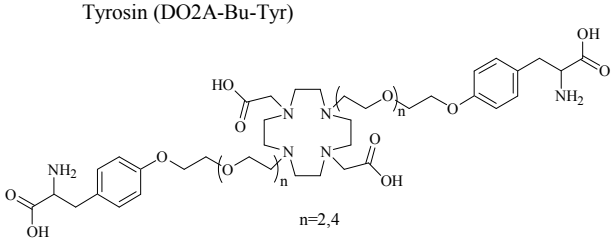

Fig.2: DO2A-tyrosine derivatives with PEG spacer

The labelling of the compounds with ^{68}Ge/^{68}Ga-generator produced ^{68}Ga was carried out in 5 ml water using 10 µg substance. This mixture was preheated to 90°C, then the volume-concentrated and purified ^{68}Ga was added [3]. The labelling-yields after 10 minutes were 65-73% for the DO2A- and over 90% for the DO3A-derivatives. After the labelling the product was retained on a SCX-cartridge and eluted in 2 ml PBS-buffer.

Evaluation: Cell studies were carried out on F98-glioblastoma cells at 0°C with ^{68}Ga-DO2A-Bu-Tyr (Fig. 3, black line). The uptake was blocked with a mixture of BCH, TRP and SER (Fig. 3, red line). The unspecific binding was determined with DO2A without any targeting vector (Fig. 3, blue line). These studies indicate a specific uptake, probably via the amino acid transporter into the cells, which can be blocked to the level of unspecific binding.

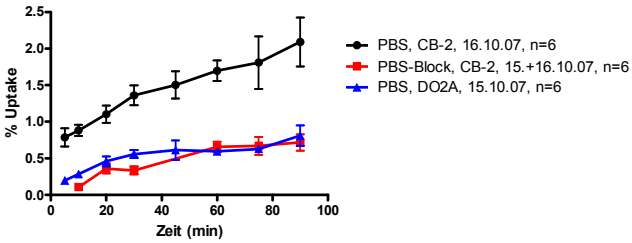

Fig.3: ^{68}Ga-DO2A-Bu-Tyr cell studies with F98-glioblastoma cells

The same substance (^{68}Ga-DO2A-Bu-Tyr) was used for a first *in vivo* small animal PET measurement. 20 MBq in 1 ml 0.9% NaCl-solution were injected to a AT1-tumor-bearing Kopenhagen rat. The tumours were placed on the feet of the rat. Tumour uptake was clearly visible. The tumour to non-tumour ratio after 60 minutes was about 1.5.

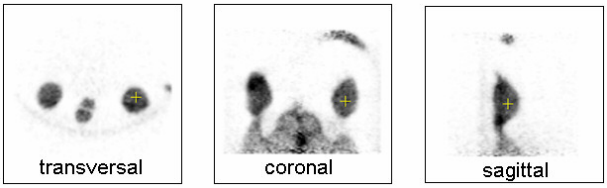

Fig.4: PET-measurement of a tumour bearing rat with DO2A-Bu-Tyr

The next steps should be the evaluation of the other substances synthesized in systematic *in vitro* cell studies, metabolite studies, an *ex vivo* distribution and further *in vivo* PET measurements.

References:

[1] H. J. Wester, Radiochemistry and Radiopharmaceutical chemistry in Life Sciences. Volume 4, chapter 5, in A Vértes, S Nagy, Z Klencsár, (eds.), Handbook of Nuclear Chemistry, 2003, Kluwer Academic Publishers, The Netherlands

[2] Z. Kovacs, A. D. Sherry, J. Chem. Soc., Chem. Commun., 1995, 185

[3] Zhernosekov KP, Filosofov DV, Baum RP, Aschoff P, Bihl H, Razbash AA, Jahn M, Jennewein M, Rosch F. Processing of generator-produced ^{68}Ga for medical application. J Nucl Med. 48 (2007) 1741-1748