^{44}Sc-DOTA-Puromycin: µPET-imaging of protein synthesis

Eigner, Sebastian[1], Beckford Vera, Denis R.[1], Fellner, Marco[2], Loktionova, Natalia S.[2], Piel, Markus[2], Melichar, František[1], Rösch, Frank[2], Roß, Tobias L.[2], Eigner Henke, Katerina[1]
[1]Radiopharmacy, Nuclear Physics Institute of the Academy of Science of the Czech Republic, Husinec, Czech Republic.
[2]Institute of Nuclear Chemistry, Johannes Gutenberg-University, Mainz, Germany

Introduction: Puromycin is an antibiotic that inhibits protein synthesis by competitive incorporation against an aminoacyl-tRNA on the ribosome A-site[1-3]. Applying pharmaceutical concentrations, premature proteins are produced. Using lower concentrations Puromycin binds specifically to the C-terminus of full-length proteins[4]. The purpose of this study was to investigate whether ^{44}Sc-labeled Puromycin can be utilized for imaging of protein synthesis *in vivo* using PET.

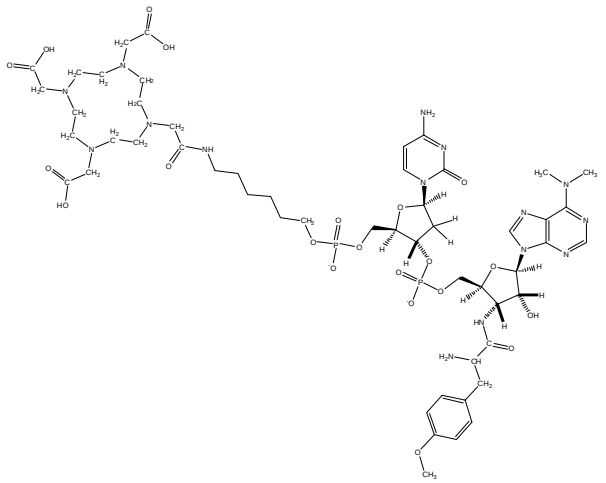

***Figure 1*:** Structure of DOTA-NHC$_6$-deoxy-cytidine-puromycin (DOTA-Pur).

Methods: DOTA-puromycin (DOTA-Pur) was synthesized using a puromycin-tethered CPG support by the usual protocol for automated DNA and RNA synthesis following our design[5] (Figure 1). ^{44}Sc was obtained from ^{44}Ti/^{44}Sc-generator as described previously by Filosofov *et al.* in 2010[6]. The generator eluate was directly used for labeling of DOTA-Pur at 95 °C for 20 minutes. The reaction mixture was passed over a C-18 cartridge and ^{44}Sc-DOTA-Pur eluted with ethanol. For µPET-studies 20-25 MBq of ^{44}Sc-DOTA-Pur was administered to tumor bearing rats via intravenous injection into a tail vein and animals were scanned for 1 hour dynamically. Specificity of the imaging was further validated by dissecting the animals after the measurement and *in vitro* blocking experiments using puromycin or cycloheximide to block protein synthesis or ribosome activity, respectively.

Results and Discussion: µPET-images of tumor bearing rats showed significant tumor uptake of ^{44}Sc-DOTA-Pur and a clear-cut tumor visualization (Figure 2). Obtained data from biodistribution showed similar results with prior conducted biodistribution and protein-incorporation studies. In both blocking experiments, cellular uptake of ^{44}Sc-DOTA-Puromycin could be totally blocked by blocking protein synthesis or ribosome activity.

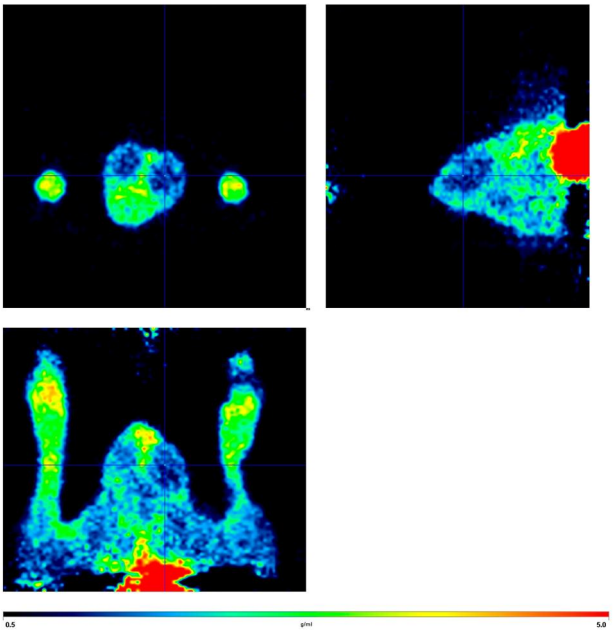

Figure 2: ^{44}Sc-DOTA-Pur accumulation in Walker carcinoma on hind feet of CD-rats.

Conclusions: We demonstrated for the first time non-invasive µPET-imaging of protein synthesis with a puromycin-based radiopharmaceutical and the direct correlation between cellular uptake of ^{44}Sc-DOTA-Pur and protein synthesis.

Acknowledgements: This project was subsidized by the Ministry of Education, Youth and Sports of the Czech Republic: Project NPV II 2B06165.

References

[1] Shelley R Starck and Richard W Roberts, Puromycin oligonucleotides reveal steric restrictions for ribosome entry and multiple modes of translation inhibition. RNA 8 (2002), 890-903.

[2] E. Miyamoto-Sato, N. Nemoto, K. Kobayashi, H. Yanagawa, Specific bonding of puromycin to full-length protein at the C-terminus; Nucleic Acids Res. 28 (2000) 1176–1182.

[3] N. Nemoto, E. Miyamoto-Sato, H. Yanagawa, Fluorescence labeling of the C-terminus of proteins with a puromycin analogue in cell-free translation systems; FEBS Lett. 462 (1999) 43–46.

[4] M. Kurz, K. Gu, P.A. Lohse, Psoralen photo-crosslinked mRNA–puromycin conjugates: a novel template for the rapid and facile preparation of mRNA–protein fusions, Nucleic Acids Res. 28 (2000) 83.

[5] Eigner S., Eigner Henke K., Beckford Vera D. R., Melichar F.: ^{86}Y-DOTA-Puromycin-Oligonucleotide: A new potential approach to in vivo quantification of protein synthesis; Eur J Nucl Med Mol Imaging 37 suppl. 2 (2010), 264.

[6] Filosofov D.V., Loktionova N.S., Rösch F.: A ^{44}Ti/^{44}Sc radionuclide generator for potential application of ^{44}Sc-based PET-radiopharmaceuticals; Radiochimica Acta, 98 (2010), 149-156.