DOTA-BN[2-14]NH_2 labeled with ^{68}Ga & ^{44}Sc PET tracers - *in vitro* and *in vivo* studies

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Introduction: In recent years, Peptide Receptor Radionuclide Therapy (PRRT) is utilizing synthetic peptides as vectors for the delivery of radionuclides such as ^{90}Y and ^{177}Lu. This has been accomplished with the aid of PET diagnostics which use the same vector molecules labeled with positron emitters. This was demonstrated at first with ^{68}Ga-labeled somatostatin analogues for diagnostics imaging of neuroendocrine tumors [1]. So far, the ^{68}Ge/^{68}Ga generator remains the most popular PET generator which is moreover commercially available from several manufacturers. From the long-lived mother ^{68}Ge ($T_{½}$ = 270.8 d) it provides ^{68}Ga ($T_{½}$ = 67.71 min) which subsequently decays to stable ^{68}Zn. Recent developments have provided a high-performance 5 mCi ^{44}Ti/^{44}Sc radionuclide generator [2, 3]. ^{44}Sc is a positron emitting radionuclide (E_{γ} 1157.0 keV, I_{γ} 99.9%, $E_{\beta+}$ 1474.3 keV, $I_{\beta+}$ 94.34%), with a half life of 3.97 h.

The present study focused on the comparison of *in vitro* and *in vivo* properties of ^{44}Sc and ^{68}Ga-labeled DOTA-functionalized bombesin (BN) analog. DOTA-BN[2-14]NH_2 (*DOTA-QRLGNQWAVGHLM-NH_2).*

Experimental: *^{68}Ga and ^{44}Sc labeling of DOTA-BN[2-14]NH_2.* For labeling of both radiometals 26.3 nmol of DOTA-BN[2-14]NH_2 was taken. Both mixtures were incubated at 95 °C for up to 25 min. *In vitro studies.* The *in vitro* studies were performed according to standard protocols and followed those used in comparative evaluation of ^{90}Y and ^{177}Lu labeled DOTA-BN[2-14]NH_2. *Biodistribution studies.* Biodistribution studies were performed in male Sprague-Dawley rats (weight 190-230 g) under pentobarbital anesthesia (40 mg/kg body weight, Narcoren, Merial, Hallbergmoos, Germany), after intravenous injection (i.v.) of the radioactive sample into the jugular vein. *Small animal PET imaging studies.* The dynamic micro-PET imaging was performed in male Copenhagen rats bearing the androgen-independent Dunning R-3327-AT-1 prostatic cancer tumor, which has been identified to express high affinity binding sites for GRP/BN analogs. Solid carcinomas of were heterotopically induced by injection of AT-1 cells (0.4 ml approx. 10^4 cells/µl) subcutaneously into the dorsum of the hind foot.

Results: *Labeling studies*. The eluted radioactivity of ^{68}Ga ranged from 100 to 150 MBq in 0.4 mL 0.05M HCl/97.56% acetone, pH 2, the respective eluted radioactivity for ^{44}Sc varied from 150 to 200 MBq in 3 mL ammonium acetate (0.25M), pH 4. The labeling yield was higher than 80% for both ^{68}Ga-DOTA-BN[2-14]NH_2 and ^{44}Sc-DOTA- BN[2-14]NH_2. The specific activity (A_s) achieved for ^{68}Ga-DOTA-BN[2-14]NH_2 was 7.5-8.0 GBq/µmol. The A_s achieved for ^{44}Sc-DOTA-BN[2-14]NH_2 was ~4.8 GBq/µmol. *In vitro studies.* ^{68}Ga-DOTA-BN[2-14]NH_2 and ^{44}Sc-DOTA-BN[2-14]NH_2 were stable up to 2h in human serum at 37 °C as analyzed by TLC. *Animal studies*. *Ex vivo organ distribution.* Both complexes showed fast blood clearance and mainly renal excretion. The main organ of uptake was pancreas due to the naturally expressed GRP receptors. The uptake of ^{68}Ga-DOTA-BN[2-14]NH_2 in pancreas was 0.64 %ID/g at 1h p.i. and 0.58±0.05 %ID/g at 2h p.i. ^{44}Sc-DOTA-BN[2-14]NH_2 showed 2.67±0.53 %ID/g at 1h p.i. and 1.51±1.19 %ID/g at 2h p.i. uptake in pancreas. The specificity of uptake was verified at 1h p.i. (0.73±0.13 %ID/g) with 73% blocking of GRP receptors in pancreas. *In vivo studies.* The *in vivo* PET imaging of ^{68}Ga-DOTA-BN[2-14]NH_2 and ^{44}Sc-DOTA-BN[2-14]NH_2 was performed in male Copenhagen rats bearing the R-3327-AT-1 prostatic cancer tumor (Figure 1).

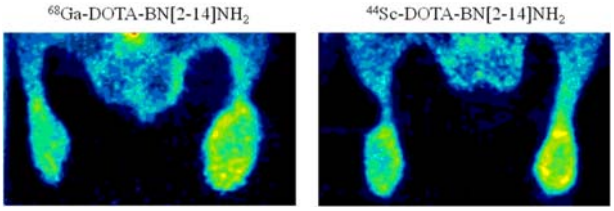

Figure 1. Cumulative µPET images (15 to 60 min p.i.) of subcutaneous R-3327- AT-1 tumors after injection of ^{68}Ga-DOTA-BN[2-14]NH_2 or ^{44}Sc-DOTA-BN[2-14]NH_2

Discussion: Considering the rather short half-life of ^{68}Ga, the ^{44}Sc can be an ideal alternative for conjugation with biomolecules of longer metabolic half-life and for acquiring extensive PET imaging studies when it is required. DOTA-BN[2-14]NH_2 can be labeled with ^{68}Ga and ^{44}Sc in a fast and efficient way based on the method established prior for the labeling of DOTA-TOC. Initial synthesis yields were higher than 80%. The radiolabeled compounds were of low specific activity in terms of activity per peptide (GBq/µmol peptide) but of adequate radioactive concentration for further *in vitro* and *in vivo* studies.

Acknowledgements

This project was supported in part by the EC via the COST action BM0607 and D38.

References

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