Development of the ^{68}Ga-labeled pan-somatostatin analog SOM230 (SOMscan®)

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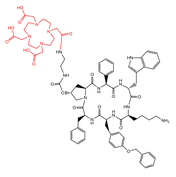
Background and aim of the study

Somatostatin receptors (sst1-sst5) are expressed on many neuroendocrine tumors. Thus, radiolabeled somatostatin analogs play an important role in targeted imaging and radionuclide therapy of these tumors. Many patients are studied nowadays with ^{68}Ga-DOTA-TOC or ^{68}Ga-DOTA-TATE, targeting sst2 [1]. Analogs with a broader receptor subtype affinity profile, such as ^{68}Ga-DOTA-NOC, targeting sst2, sst3 and sst5, may image a broader spectrum of tumors or may increase the net tumor uptake, given the presence of several receptor subtypes on the same tumor cell. SOM230 (Pasireotide) binds to human sst1, 2, 3, and 5 and mimics the action of natural somatostatin [2].

The **aim** of our study is to develop a ^{68}Ga-labeled PET imaging probe based on SOM230 (Pasireotide) targeting a broad spectrum of somatostatin receptors for clinical application.



DOTA-SOM230

Radiotracer

The somatostatin analog SOM230 was synthesized using solid phase peptide synthesis (Novartis, Basel, Switzerland). The macrocyclic chelators DOTA was coupled and the conjugate DOTA-SOM230 were purified by preparative RP-HPLC and identified by ESI-MS (piChem, Graz, Austria).

Labeling with ^{68}Ga took place in sodium acetate buffer 0.2 mol/L, pH 4.0 using the Modular-Lab PharmTracer module by Eckert & Ziegler (Berlin, Germany). The labeled peptide ^{68}Ga-DOTA-SOM230 was obtained in a radiochemical purity of ≥ 92% and in a specific activity of 50 GBq/µmol.

In vitro evaluation

The **affinity** of the (metallo)peptides was determined by competition binding experiments on membrane preparations from HEK-hsst1, -hsst2, -hsst3 and -hsst5. The half-maximal inhibitory concentrations (IC_{50}) were estimated by displacement using the somatostatin radioligand ^{125}I-[Tyr^{11}]-SS-14 in the present or absence of each (metallo)peptide in concentrations ranging from 10^{-12} to 10^{-6} M. The natural somatostatin-14 (SS-14) was used as control. Data are presented in Table 1.

Table 1. IC_{50} values in nmol/L (mean ± SEM; n = 3-7) for human sst1, sst2, sst3 and sst5

Compound	hsst1	hsst2	hsst3	hsst5
SS-14	21.8 ± 7.7	5.7 ± 1.1	5.1 ± 1.7	22.7 ± 3.5
SOM230	31.3 ± 11.1	21.3 ± 5.7	38.7 ± 11.7	3.6 ± 1.5
DOTA-SOM230	4.4 ± 1.8	5.1 ± 1.3	9.8 ± 0.71	0.3 ± 0.1
^{nat}Ga-DOTA-SOM230	2.1 ± 0.3	4.4 ± 0.7	3.3 ± 1.3	0.3 ± 0.1

References

[1] Maecke HR, Reubi JC. *J Nucl Med.* **2011**; 52(6): 841-4.

[2] Bruns C, Lewis I, Briner U, Meno-Tetang G, Weckbecker G. *Eur J Endocrinol.* **2002**; 146(5): 707-16.

Immunocytochemistry was performed on HEK-hsst2, HEK-hsst3 and HEK-hsst5 cells. After the appropriate treatment with SS-14 or DOTA-TATE (controls), SOM230 and ^{nat}Ga-DOTA-SOM230, cells were fixed with 4% paraformaldehyde and 0.2% picric acid in phosphate buffer (pH 6.9) for 30 min at room temperature and washed several times. Specimens were permeabilized and then incubated with anti-sst2 {UMB-1}, anti-sst3 {UMB-5} or anti-sst5 {UMB-4} antibodies followed by Alexa488-conjugated secondary antibodies (Amersham, Braunschweig, Germany). Specimens were mounted and examined using a Zeiss LSM510 META laser scanning confocal microscope.

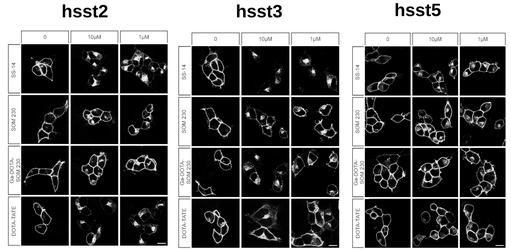
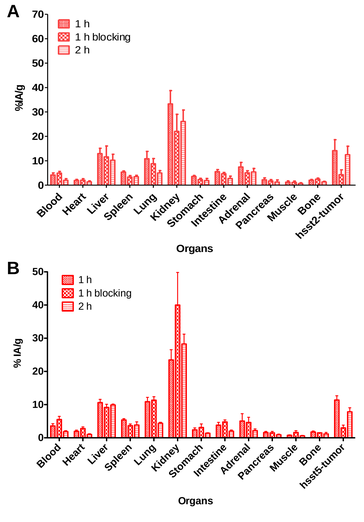


Figure 1. Representative images of internalization in HEK cells stably expressing human sst2 (left panel), sst3 (middle panel) or sst5 (right panel) receptors after treatment with either 0, 1 µM or 10 µM SS-14, SOM230, ^{nat}Ga-DOTA-SOM230 or DOTA-TATE. *Scale bar*: 20 µm.

In vivo evaluation

Biodistribution and **small-animal PET** imaging studies were performed in nude mice bearing HEK-sst2 and HEK-hsst5 tumor xenografts. Mice were injected with 100 µL/100 pmol ^{68}Ga-DOTA-SOM230 via the tail vein and were euthanized at 1 and 2 h p.i. Non-specific uptake was determined 1 h p.i. with co-injection of 1500-fold excess of DOTA-SOM230. Clinically used radiotracers were evaluated in parallel as reference compounds, namely ^{68}Ga-DOTA-TATE in hsst2 xenografts and ^{68}Ga-DOTA-NOC in hsst5 xenografts.



Ratio	1 h	2 h
Tumor : Blood	2.6 ± 0.7	4.0 ± 1.6
Tumor : Liver	1.1 ± 0.3	1.2 ± 0.3
Tumor : Kidney	0.5 ± 0.1	0.5 ± 0.2
Tumor : Muscles	11.9 ± 3.9	12.7 ± 5.6

Ratio	1 h	2 h
Tumor : Blood	3.3 ± 0.5	4.2 ± 0.4
Tumor : Liver	1.1 ± 0.0	0.8 ± 0.1
Tumor : Kidney	0.5 ± 0.1	0.3 ± 0.0
Tumor : Muscles	15.8 ± 2.3	13.4 ± 2.5

Figure 2. Biodistribution results of ^{68}Ga-DOTA-SOM230 in HEK-**hsst2 (A)** and HEK-**hsst5 (B)** tumor xenografts at 1 and 2 h p.i., along with blocking experiments 1 h p.i. Data are expressed as %IA/g ± SD (n = 4-7).

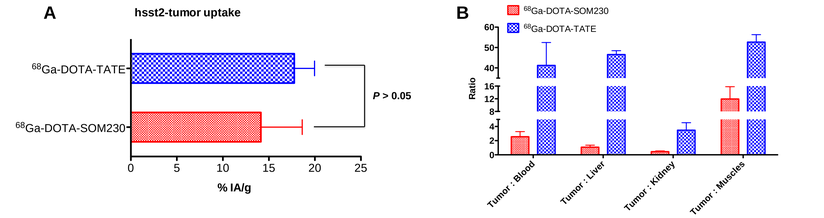


Figure 3. A) Tumor uptake of ^{68}Ga-DOTA-SOM230 in HEK-**hsst2** xenografts is slightly lower (14.1 ± 4.5 %IA/g) that the reference compound ^{68}Ga-DOTA-TATE (17.8 ± 2.2 %IA/g) 1 h p.i. but statistically not significant (P > 0.05). **B)** The tumor-to-normal tissue ratios of ^{68}Ga-DOTA-TATE are significantly higher than ^{68}Ga-DOTA-SOM230.

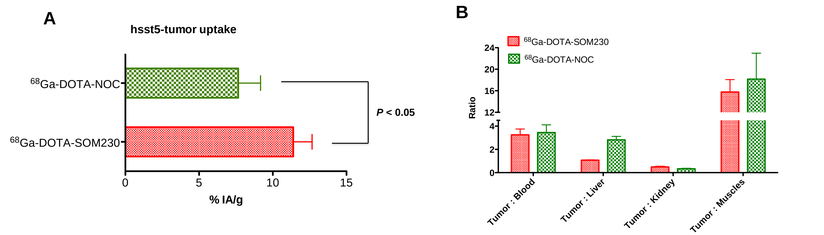


Figure 4. A) Tumor uptake of ^{68}Ga-DOTA-SOM230 in HEK-**hsst5** xenografts (11.4 ± 1.3 %IA/g) is statistically significant higher that the reference compound ^{68}Ga-DOTA-NOC (7.7 ± 1.5 %IA/g) 1 h p.i. (P < 0.05). **B)** The tumor-to-normal tissue ratios of ^{68}Ga-DOTA-SOM230 is comparable to ^{68}Ga-DOTA-NOC.

Small-animal PET images

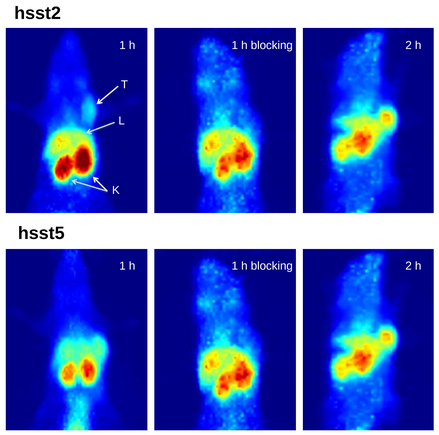


Figure 5. Small-animal PET images (coronal sections) of ^{68}Ga-DOTA-SOM230 in HEK-hsst2 (upper panel) and HEK-hsst5 xenografts (lower panel) at 1 and 2 h p.i. along with blocking at 1 h p.i. The images clearly visualized the tumors, they showed high kidney and relatively high liver uptake (T: tumor, L: liver, K: kidneys) and prove the specificity of the radiopeptides for the hsst2 and hsst5 receptors, as no tumor is visualized after co-injection of excess of unlabeled peptide.

Conclusion

The initial evaluation of ^{68}Ga-DOTA-SOM230 (SOMscan®) reveals its potentiality as PET probe for *in vivo* imaging of a broad spectrum of somatostatin receptors.