Synthesis and characterization of a ^{68}Ga/NIR labeled peptide for somatostatin receptor targeting

Sukhen C. Ghosh[1], Servando Hernandez Vargas[1], Julie Voss[1], Dongyoul Lee[2], Michael K. Schultz[2], Ali Azhdarinia[1]

The University of Texas Health Science Center at Houston – McGovern Medical School, [1]Institute of Molecular Medicine and [2]Department of Radiology, University of Iowa

Objectives

Fluorescently labeled imaging agents can identify surgical margins in real-time to help achieve complete resections and minimize the likelihood of local recurrence. However, photon attenuation limits fluorescence-based imaging to superficial lesions or lesions that are few millimeters beneath the tissue surface. Contrast agents that are dual-labeled with a radionuclide and fluorescent dye can overcome this limitation and combine quantitative, whole-body nuclear imaging with intraoperative fluorescence imaging. An ideal approach for dual labeling would be to use a clinically established radiotracer since it could provide a benchmark to characterize the dual-labeled counterpart. Here, we used a modular dual labeling scaffold, referred to as a multimodality chelator (MMC), to develop a fluorescent ^{68}Ga-DOTA-TOC analog and evaluated agent properties *in vitro* and *in vivo*.

Methods

In order to develop a fluorescent ^{68}Ga-DOTA-TOC analog with minimal structural deviations, we functionalized the macrocyclic compound 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid (DO2A) with two different pendant arms to enable modular synthesis. The resulting MMC was conjugated to the N-terminus of Tyr3-octreotide (TOC) on solid-phase. IRDye800 was then conjugated to an azido group on the second arm by click chemistry to produce MMC(IR800)-TOC. ^{68}Ga labeling methods were optimized and used to prepare cold-labeled probes. *In vitro* studies for determining receptor-targeting properties as well as cellular uptake/ blocking of cold-labeled/radiolabeled analogs were investigated -and compared to ^{68}Ga-DOTA-TOC- in HEK-293 cells and HCT116 cells that stably overexpress SSTR2 (HCT116-SSTR2). PET/CT and near-infrared fluorescence (NIRF) imaging was conducted in HCT116(SSTR2) xenografts at 1, 3, and 24 h post-injection.

Results

The MMC scaffold permitted efficient production of ^{68}Ga-MMC(IR800)-TOC (radiochemical yield: 79.9±8.1%, specificity activity: 87.3 TBq/mmol). Receptor-pharmacology assays revealed that Ga-MMC(IR800)-TOC retains intact agonist properties. Blocking studies showed nearly complete elimination of uptake. These findings were in agreement with the radioactive uptake studies which showed similar uptake of ^{68}Ga-MMC(IR800)-TOC and ^{68}Ga-DOTA-TOC in HCT116-SSTR2 cells (25.0±1.7% vs. 21.5±3.7%, respectively), and a 93.7±1.6% reduction in ^{68}Ga-MMC(IR800)-TOC uptake when incubated with a 100-fold excess of octreotide (P < 0.0001). No notable uptake was seen in HCT116-WT cells. *In vivo*, ^{68}Ga-MMC(IR800)-TOC was observed to accumulate in tumors over time (3 h vs 24 h). The kidneys were identified as the primary excretion route for both agents. Dye labeling did cause higher clearance *via* reticuloendothelial system (RES) organs (liver and spleen) and longer retention in blood, though these values decreased rapidly over time. Washout patterns were similar in most of the remaining tissues.

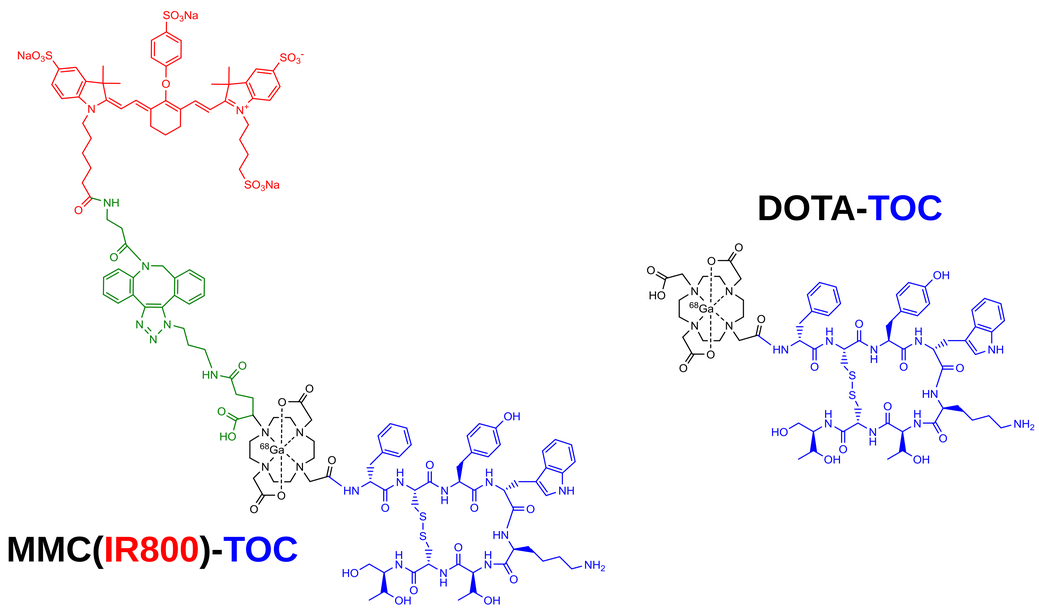
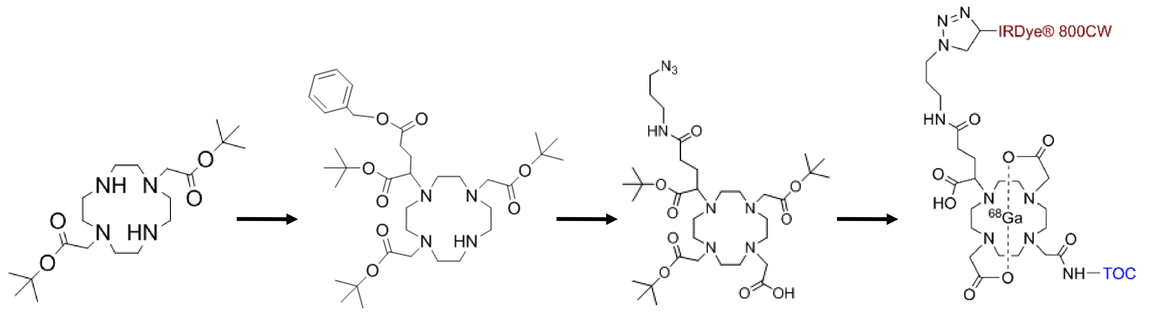


Figure 1. Chemical structures of peptide conjugates.



Scheme 1. Synthesis of ^{68}Ga-MMC(IR800)-TOC: a) modified L-glutamic acid γ-benzyl ester, conjugation of acetate and azido pendant arms, TOC conjugation, deprotection, IRDye800 conjugation, ^{68}Ga labeling..

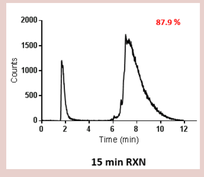
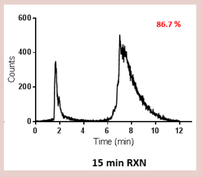
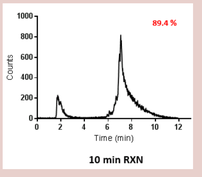
Fractionation	Acetone	NaCl
N/A	98% acetone/0.02 M HCl (v/v)	5M NaCl/5.5M HCl
2.0 M NaOAc buffer, pH=4.0	2.0 M NaOAc buffer, pH=4.0	2.0 M NaOAc buffer, pH=4.0
~380 µCi	~1 mCi	~1 mCi
		
87.9 %	86.7 %	89.4 %
15 min RXN	15 min RXN	10 min RXN

Figure 2. ^{68}Ga-MMC(IR800)-TOC labeling efficiency using clinically relevant radiochemistry formulations: eluate fractionation and two cation exchange methods (acetone and NaCl).

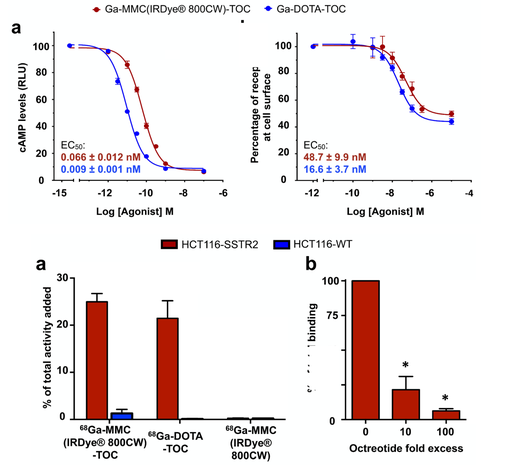


Figure 3. *In vitro* analysis of agonist properties (top) and ccellular uptake (bottom) of peptide conjugates in HEK-293 and HCT116-SSTR2 cell lines, respectively. *P < 0.0001.

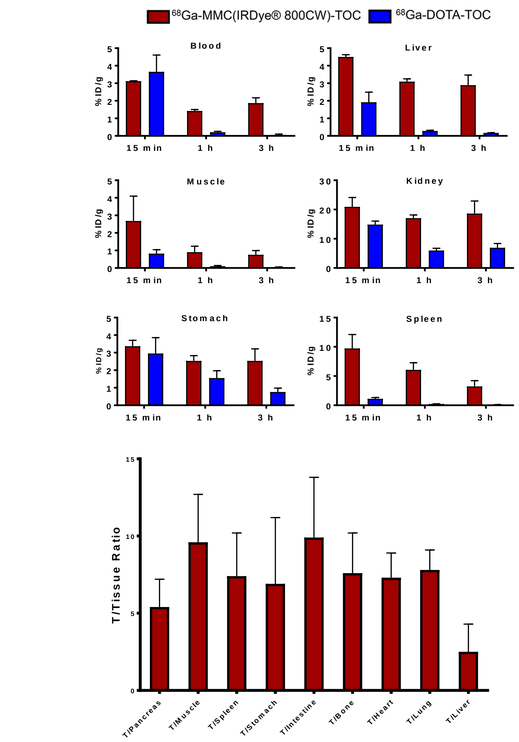


Figure 4. Biodistribution of ^{68}Ga-MMC(IR800)-TOC and ^{68}Ga-DOTA-TOC in healthy mice (top). T/Tissue ratios obtained from Ex Vivo images (24 h) using an IVIS® Spectrum Imaging System.

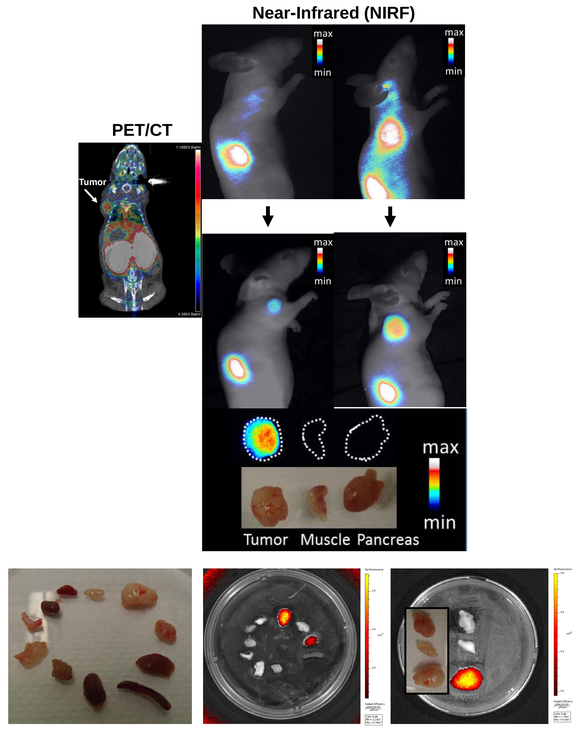


Figure 5. Multimodality images of HCT116-SSTR2 xenografts injected with ^{68}Ga-MMC)IR800)-TOC. NIRF imaging was performed with a custom EMCCD camera (top) and an IVIS Lumina XR (bottom).

Conclusions

A fluorescent ^{68}Ga-DOTA-TOC analog was synthesized using the MMC scaffold. The combination of excellent specificity for SSTR2-expressing cells and suitable biodistribution indicate potential application for intraoperative detection of SSTR2-expressing tumors.

References

1. Ghosh SC, Hernandez Vargas S, Rodriguez M, et al. Synthesis of a Fluorescently Labeled 68Ga-DOTA-TOC Analog for Somatostatin Receptor Targeting. *ACS Med Chem Lett.* 2017;8:720-725.
2. Ghosh SC, Rodriguez M, Carmon KS, et al. A Modular Dual Labeling Scaffold That Retains Agonistic Properties for Somatostatin Receptor Targeting. *J Nucl Med.* 2017

This work was supported by NIH R01EB017279 (Azhdarinia).