Image-guided delivery of temozolomide to SSTR2 expressing cells

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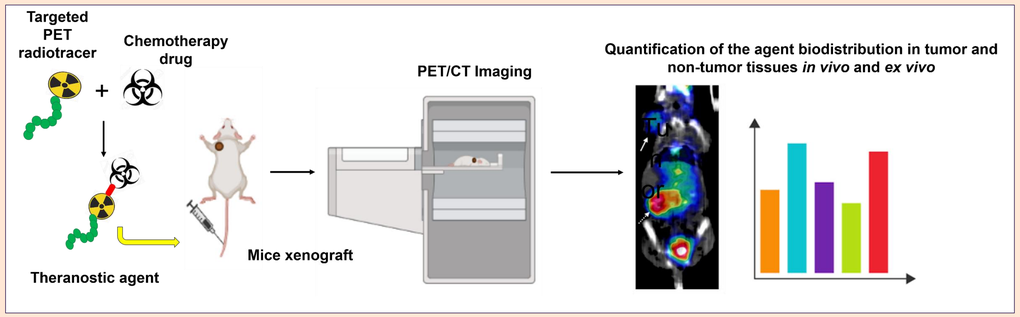
Study overview

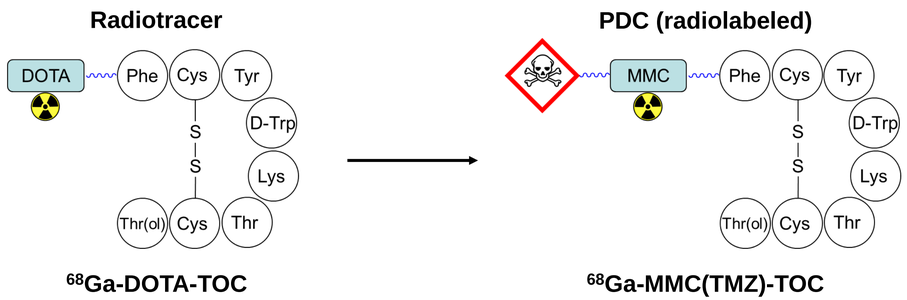


Abstract

Temozolomide (TMZ) is a DNA damaging agent that produces high response rates in neuroendocrine tumors (NETs) when the DNA repair enzyme, known as O^6-methylguanine DNA methyltransferase (MGMT), is inactivated. When given at high doses, TMZ therapy can exhaust MGMT activity and its associated resistance mechanisms, but also produces dose-limiting toxicities. Since nearly all NETs overexpress the somatostatin receptor subtype 2 (SSTR2), we hypothesized that a receptor-targeted TMZ analog could produce high intratumoral drug concentrations while avoiding systemic toxicity. Accordingly, we developed a radiolabeled peptide-drug conjugate (PDC) for SSTR2-targeted delivery of TMZ and report on the utility of the radioactive label for characterizing receptor-binding properties, pharmacokinetics, and tissue biodistribution.

Synthesis of MMC(TMZ)-TOC

The clinically used somatostatin analog, ^{68}Ga-DOTA-TOC, was used as a model for development of the SSTR2-targeted peptide drug conjugate (PDC). Synthesis was performed by (i) replacing DOTA with a multimodality chelator (MMC), (ii) attaching a modified TMZ analog to MMC, and (iii) conjugating the payload moiety to TOC on solid-phase.



MMC(TMZ)-TOC retained target specificity and internalization properties after binding

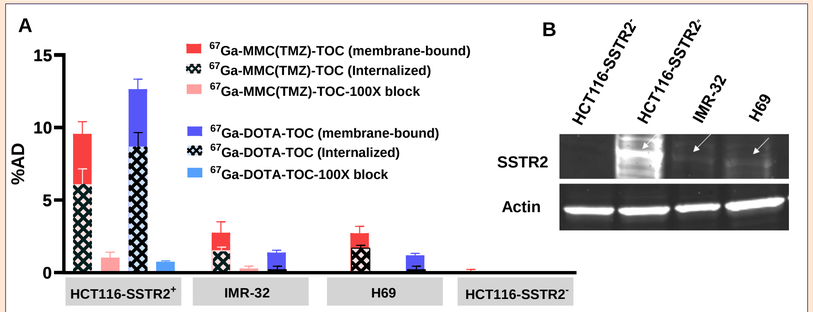


Fig 1. MMC(TMZ)-TOC retains SSTR2-targeting specificity and agonist properties. A) MMC(TMZ)-TOC was radiolabeled with ^{67}Ga to examine retention of SSTR2 binding and internalization in cell lines with different expression levels of SSTR2 in the presence and absence of the blocking agent (octreotide). Receptor-mediated uptake is shown in SSTR2-expressing cells while absent in SSTR2-negative HCT116-WT cells. Uptake was reduced in the presence of a 100-fold excess of octreotide, showing binding specificity. %AD: percentage added dose. **B)** western blot studies confirm different expression levels of SSTR2 in the tested cell lines. The uptake of the radiolabeled agent was in correlation with the amount of receptor expression.

MMC(TMZ)-TOC inhibited cell growth in SSTR2-positive cells and induced receptor-mediated DNA damage

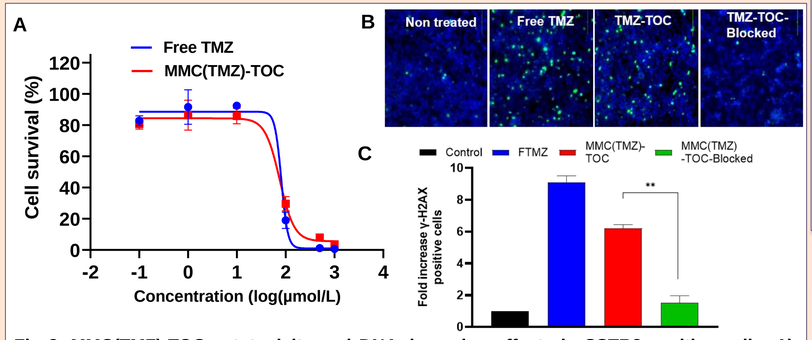


Fig 2. MMC(TMZ)-TOC cytotoxicity and DNA-damaging effects in SSTR2-positive cells. A) SSTR2$^+$ IMR-32 cells were exposed to the same dose range of each free-TMZ and MMC(TMZ)-TOC and the cell viability studies revealed that MMC(TMZ)-TOC inhibited cell growth in a dose-dependent manner with a potency similar to free TMZ. **B)** Immunofluorescent staining of ɣH2AX showed DNA double strand breaks in IMR-32 cells treated with equimolar concentrations of fTMZ and MMC(TMZ)-TOC in the presence and absence of blocking. **C)** Analysis the of the ɣH2AX foci showed 9 and 6-fold increase in the number of ɣH2AX positive cells in the free TMZ and MMC(TMZ)-TOC treated cells, respectively, compared to non-treated controls. ɣH2AX foci formation was significantly reduced in the cells co-incubated with MMC(TMZ)-TOC and blocking agent ($P<0.05$).

MMC(TMZ)-TOC showed receptor specificity in receptor-positive xenografts

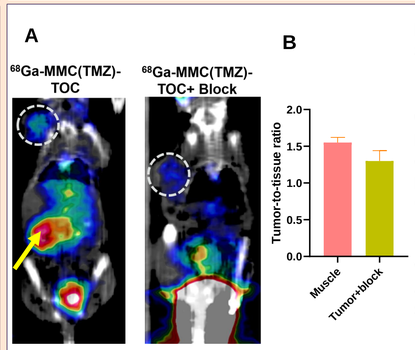


Fig. 3. *In vivo* imaging in a low SSTR2-expressing model. (A) PET/CT imaging with ^{68}Ga-MMC(TMZ)-TOC in H69 xenografts demonstrated the tumor targeting properties of the conjugate while also showing blocking in the presence of excess octreotide (white circles indicate tumors). Elimination was primarily through the kidneys (yellow arrow). Images were acquired at 1 h p.i. **(B)** Analysis of tumor to muscle ratios shows the feasibility of SSTR2-mediated drug delivery in a low-expressing xenograft model (> 1.5) and reduced tumor uptake in blocking studies.

Biodistribution analysis with a therapeutic dose of MMC(TMZ)-TOC predicted safety and efficacy

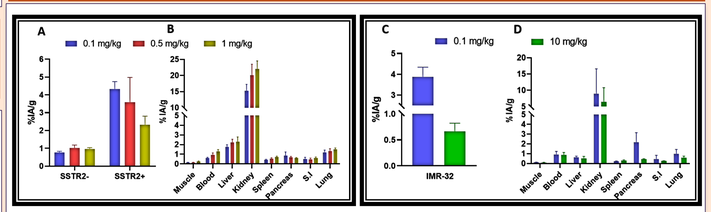


Fig. 4. Dose escalation and in vivo biodistribution of ^{67}Ga-MMC(TMZ)-TOC. *Ex vivo* biodistribution analysis via gamma counting 3 h postinjection of 0.1, 0.5, 1 or 10 mg/kg radio-conjugate in HCT116-SSTR2+/- and IMR-32 xenofrafts showing **(A and C)** specific tumor uptake and **(B and D)** low non-specific uptake in non-tumor tissues even at higher doses, except for kidney, which represents the elimination route.

Conclusions

We developed a novel drug conjugate that selectively causes toxicity and DNA damage in SSTR2-expressing cells. We showed that direct radiolabeling of MMC(TMZ)-TOC *via* the MMC allowed us to quantify the binding and internalization and compare agent performance to the gold standard, $^{67/68}$Ga-DOTA-TOC. Direct radiolabeling of MMC(TMZ)-TOC also provided quantitative evidence of SSTR2 mediated binding and biodistribution analysis in animal models. These findings demonstrate the utility of developing a radiolabeled drug conjugate and may guide optimization strategies.

References

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