Peptide-photosensitizer conjugates for implementation in GLP1R- and SSTR-targeted photodynamic therapy of neuroendocrine tumors

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Introduction

- Radical resections of neuroendocrine tumors are often associated with morbidity, and residual disease leads to recurrences. Techniques that lead to more specific and less invasive ablation of tumor cells are warranted.
- In targeted photodynamic therapy, a photosensitizer is delivered to tumor cells by coupling it to a molecule that binds a receptor that is specifically (over)expressed on tumor cells. Upon activation with near infrared light, the photosensitizer induces production of reactive oxygen species and subsequent cell death.
- Two G-protein coupled receptors that are regularly overespressed on neuroendocrine tumors are the glucagon-like peptide 1 receptor (GLP1R) and the somatostatin receptor 2 (SSTR2).
- GLP1R- and SSTR2-targeted photodynamic therapy (Figure 1) is therefore an interesting treatment option for specific and less invasive ablation of neuroendocrine tumors.

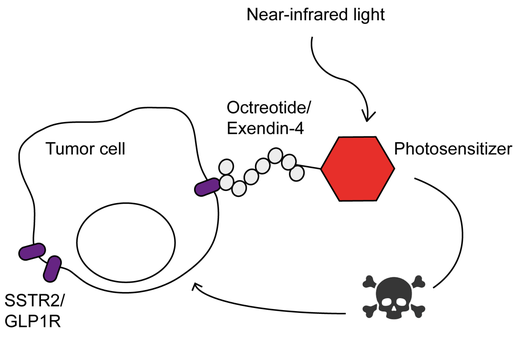


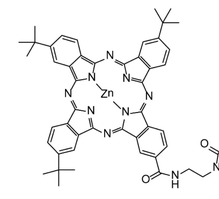
Figure 1. Targeted photodynamic therapy with peptide-photosensitizer conjugates. Upon binding to the tumor target and illumination with light of a specific wavelenght, the photosensitizer induces formation of the toxic reactive oxygen species and subsequent cell death.

Goal of this study is to develop and preclinically characterize effectivity of peptide-photosensitizer conjugates for GLP1R- and SSTR2-targeted photodynamic therapy.

Methods

- Zinc-phthalocyanine based photosensitizers TT1-maleimide (Figure 2A) and NETCure2-maleimide (Figure 2B) were synthesized and conjugated to a sulfhydryl group on the primary amine of Lys40 in exendin-4 and of dPhe in octreotide.

A



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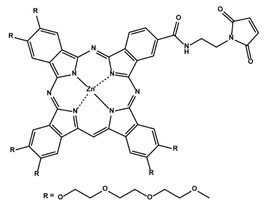

Figure 2. Structures of zinc-phthalocyanine based photosensitizers TT1 (A) and NETCure2 (B) functionalized with a maleimide for conjugation to exendin-4 and octreotide.

- **Absorbance and emission spectra** of the conjugates in dimethyl sulfoxide (DMSO), phosphate buffered saline (PBS) and PBS with bovine serum albumin (BSA) were determined.
- To determine **half-maximal inhibitory concentrations (IC_{50})**, chinese hamster lung cells stably transfected with GLP-1R (CHL-GLP1R) or SSTR-overexpressing AR42J cells were co-incubated with either exendin-4-photosensitizer conjugates and ^{111}In-labeled exendin-4-DTPA as a competitor, or octreotide-TT1 and ^{111}In-labeled octreotide-DTPA as a competitor, respectively.
- **Light-induced toxicity** by exendin-4-TT1 and exendin-4-NETCure2 was determined using CHL-GLP1R, insulinoma cells with physiological GLP1R expression (INS1) and cells without GLP1R expression (PANC1). Cells were incubated with increasing concentrations of the exendin-4 conjugates for 4 hours, and 24 hours after illumination with 690 nm light, cell viability was determined (Cell titer glo).
- **Biodistribution** of exendin-4-TT1 was determined in BALB/c nude mice carrying subcutaneous xenorgafts of CHL-GLP1R tumors. Mice were injected with 20 µg of the conjugate, and uptake of the tracer in liver, spleen, kidneys, blood, pancreas and tumor was determined *ex vivo* using fluorescence quantification in tissue homogenates as described in Boss et al (1).

Results

- Spectral properties of TT1 and NETCure2 were retained after conjugation to both peptides (Figure 3)

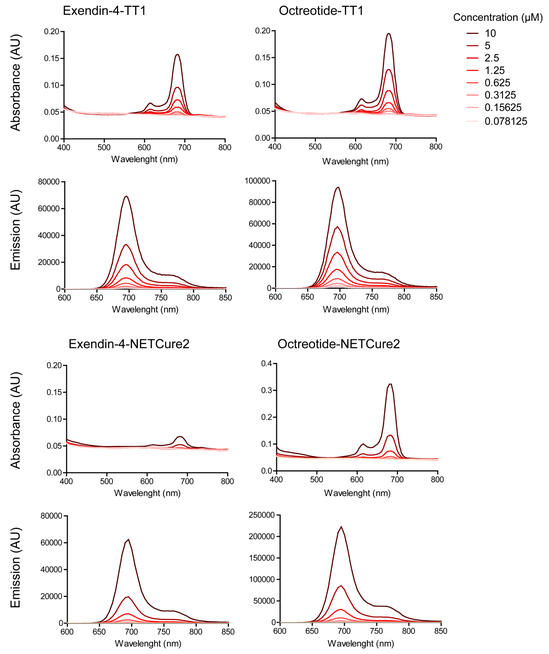

Figure 3. Absorbance and emission spectra of various concentrations of exendin-4-TT1 and octreotide-TT1 conjugates in DMSO

- Exendin-4-TT1, exendin-4-NETCure2 and octreotide-TT1 bound to receptor overexpressing cells with high affinity (0.23 µM, 0.46 µM and 0.12 µM, respectively) (Figure 4).

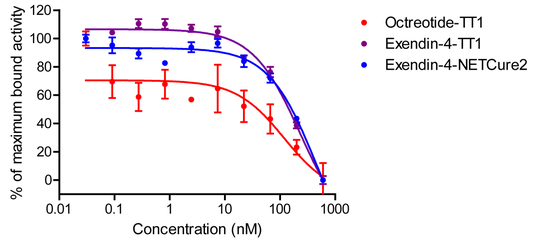

Figure 4. Competition of AR42J associated ^{111}In-labeled octreotide-DTPA by octreotide-TT1 or CHL-GLP1R associated ^{111}In-labeled exendin-4-DTPA by exendin-4-TT1 or exendin-4-NETCure2

- Exendin-4-TT1 induced concentration dependent cell death of receptor expressing cells upon illumination, while exendin-4-NETCure2 induced cell death of only CHL-GLP1R which was not peptide dose dependent (Figure 5).

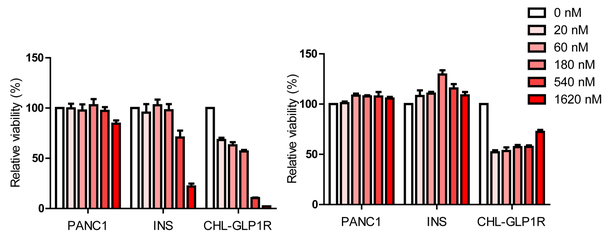

Figure 5. Cell viability of PANC1 (GLP1R negative), INS (physiological GLP1R levels) and CHL-GLP1R (overexpression of GLP1R) upon incubation with exendin-4-TT1 and illumination with 690 nm light.

- Exendin-4-TT1 is taken up in CHL-GLP1R expressing tumors *in vivo* at 4 and 18 hours post injection. Non-target specific uptake is found in the liver and spleen (Figure 6).

Figure 6. Biodistribution of 20 µg exendin-4-TT1 at 4 and 16 hours post intravenous injection in mice carrying subcutaneous CHL-GLP1R xenografts. Uptake is depicted as percentage of the injected peptide dose per gram of tissue (%ID/g)

Conclusions and discussion

In conclusion, the novel photosensitizer-peptide conjugates show potency for SSTR2- and GLP1R-targeted photodynamic therapy

In ongoing studies, we will:

- Characterize in vitro activity of the octreotide-NETCure2 conjugates
- Determine in vivo targeting and light induced ablation of target-expressing tumor cells by all conjugates

References

1. Boss M et al. Receptor-targeted photodynamic therapy of glucagon-like peptide 1 receptor positive lesions. Journal of Nuclear Medicine 2020

Acknowledgements

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