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Pre-Clinical evaluation of a Novel [^{18}F]-labeled d-TCO amide derivative for Bioorthogonal Pretargeted Imaging of Cancer

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Objectives

Bioorthogonal chemistry has found rapidly growing applications in the field of molecular imaging. Considerable research has been devoted to the development of antibody-trans-cyclooctene (TCO) conjugates, and their application for pretargeted tumor imaging has been reported under different modalities. However, it has been shown that TCO has the tendency to isomerize to its isomer, cis-cyclooctene (CCO), after prolonged exposure to physiological conditions. Given this, we envisaged a different approach where the development of more stable [18F]-labeled TCOs is investigated for pretargeted PET imaging using a tetrazine-modified antibody.

Methods

Synthesis and characterization by NMR and high-resolution mass spectrometry of all compounds was performed. Antibody CC49 (anti TAG-72) was modified with an "in house" tetrazine [1] and analyzed by SDS-PAGE and mass spectrometry to determine the degree of labeling. Rate constants for the reaction between d-TCO and tetrazine were measured by stopped-flow under aqueous conditions. Tracer in vitro stability was evaluated in PBS and mouse plasma at 37°C. In vivo and ex vivo biodistribution studies were performed in nude mice by IV administration of 0.25 mCi of tracer and a whole-body dynamic (0-60min postinjection) scans were acquire. In vivo and ex vivo PET/CT pretargeted imaging was carried out in human colorectal tumor xenografts where CC49-tetrazine (150µg/100µl) and CC49 (100µg/100µl; control) were administered IV followed 24h later by [18F]d-TCO (0.25 mCi) injection. Whole-body (60min postinjection) static scan was acquired.

Results

The novel d-TCO reacted with the tetrazine at a rate constant (k_2) of 10 553 $M^{-1}s^{-1}$. The radiolabeling of d-TCO with [18F] provided a radiochemical yield (RCY) of 5.5±0.5 % (decay corrected to EOB) and > 98% radiochemical purity. Tracer presented a moderate lipophilicity with a log*D* value 1.73±0.01. Tracer showed no isomerization after incubation with PBS and plasma up to 2h and 1h, respectively. In vivo stability study showed that 25% of the tracer was intact at 5min p.i. In control mice the tracer showed a mixed hepatobiliary and renal clearance, displaying at 2h p.i 0.44±0.03 %ID/g in the blood, 4.85±0.17 %ID/g in the small intestine, 5.05±0.20 %ID/g in the large intestine and 1.31±0.09 %ID/g in the kidneys.

In tumor xenografts the average uptake in the tumors was 2.1±0.7 %ID/g at 60min p.i. when pre-injected with CC49-tetrazine and 0.7±0.1 %ID/g p.i. when pre-injected with CC49 (control). A higher tumor uptake could be observed when the mice were treated with CC49-tetrazine when compared with the control. Furthermore, tumor-to-muscle ratio was 2.5±0.11.

Conclusions

We developed a new [18F]-d-TCO tracer that showed good stability with fast reaction kinetics towards tetrazine and a favorable pharmacokinetic profile for pretargeting approaches.

We were also able to demonstrate the successful use of a new [18F]-labeled d-TCO in a PET pretargeted imaging approach, allowing visualization of the tumor with a significant higher uptake when compared to the control. With this approach we are able to avoid the prolonged

exposure of TCO to plasma proteins leading to its deactivation. Furthermore, thinking about future *in vivo* applications having a more hydrophobic-labeled TCO can bring advantages when cell membranes and blood-brain barrier need to be crossed.

References

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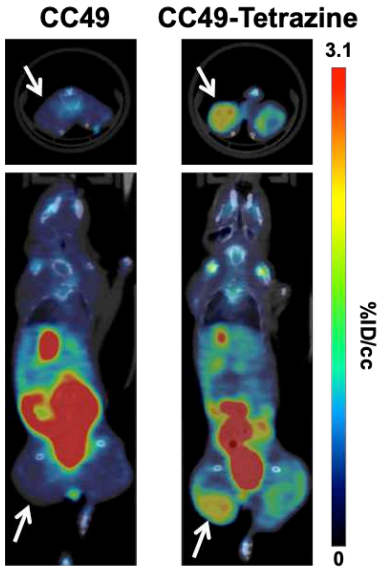

Fig.1 - Pretargeted PET/CT imaging of tumor-bearing mice 1h after injection of [18F]d-TCO.