|  |
| --- |
| **Transcyclooctene-modified PEGylated liposomes and a tris(hydroxypyridinone)-tetrazine conjugate for pretargeted PET imaging of liposomal nanomedicines** |
| Aishwarya Mishra1, Amaia Carrascal-Minino1, Jana Kim1, Rafael T.M. de Rosales1 |
| 1School of Biomedical Engineering & Imaging Sciences, King's College London, London, United Kingdom. |
| **Background:** Transcyclooctene and tetrazine are an effective pair for establishing in vivo biorthogonal chemistry systems. These systems have been effectively used to develop pretargeted imaging of therapeutic monoclonal antibodies with valuable advantages over conventional imaging. This pretargeted imaging can also be applied to long blood circulating PEGylated nanomedicines enabling decreased radiation doses to patients during longitudinal imaging and radiotherapy. Here, we report synthesis of TCO-modified liposomes and radionuclide Ga-chelating THP-tetrazine and their pretargeting validation in vitro and in vivo. |
| **Methods:** THP-tetrazine was synthesized via reaction of THP-Bz-SCN with Methyl tetrazine amine. The reaction mixture was purified and characterized with LCMS/NMR. A TCO-PL conjugate was synthesized via conjugation of DSPE-PEG(2k)-NH2 (PL) to TCO-NHS ester. TCO-PL was purified using dialysis and characterized by NMR/HRMS. TCO-PL was inserted into the bilayer of PEGylated liposomes (Lip) by co-incubating TCO-PL and Lip to give TCO-PL-Lip. In vitro validation of biorthogonal reaction was performed by co-incubating 68Ga-THP-tetrazine and TCO-PL-lip in serum. In vivo imaging and biodistribution were performed on healthy mice and fibrosarcoma tumor model: test group (TCO-PL-lip i.v. followed by 68Ga-THP-tetrazine i.v.), positive control (67Ga-THP-PL-lip i.v.) and negative control (68Ga-THP-tetrazine i.v.). |
| **Results:** The structure of synthesized THP-tetrazine was confirmed by NMR/LCMS. The radiolabeling of 68Ga confirmed that chelating properties of THP have been retained by THP-tetrazine. The synthesized TCO-PL was incorporated in the bilayer of the PEGylated liposomes providing a reactive site for radiolabeled THP-tetrazine via biorthogonal reaction. The biorthogonal reactive pair TCO-PL-lip and 68Ga-THP-tetrazine exhibited fast reaction kinetics in vitro in PBS and serum. In vivo validation in healthy and tumor mice confirmed the biorthogonal pretargeting was observed at 24 hr post liposomal administration showing high uptake in spleen and liver compared to negative control. However, the observed tumor uptake for the pretargeting test group is much lower compared to positive control. |
| **Conclusions:** The biorthogonal pair: THP-tetrazine and TCO-PL-Lip were successfully synthesized and characterized. The presence of TCO chelators on the liposomal surface allows for fast reaction with 68/67Ga-THP-tetrazine in the presence of human serum and ions in vitro. In vivo labelling of TCO-liposomes is observed in healthy and tumour mice showing high pretargeting in liver and spleen but limited pretargeting in tumored animals. |