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Characterization of Dota-Girentuximab Conjugates for Radioimmunotherapy

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Abstract : Radiopharmaceuticals based in monoclonal anti-body (mAb) via chemical linkers have become a potential tool in nuclear medicine because of their specificity and the large variability and availability of therapeutic radiometals. It is important to identify the conjugation sites and number of attached chelator to mAb to obtain radioimmunoconjugates with required immunoreactivity and radiostability. Girentuximab antibody (G250) is a potential candidate for radioimmunotherapy of clear cell carcinomas (RCCs) because it is reactive with CAIX antigen, a transmembrane glycoprotein overexpressed on the cell surface of most (> 90%) (RCCs). G250 was conjugated with the bifunctional chelating agent DOTA (1,4,7,10-Tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid) via a benzyl-thiocyano group as a linker (p-SCN-Bn-DOTA). DOTA-G250 conjugates were analyzed by size exclusion chromatography (SE-HPLC) and by electrophoresis (SDS-PAGE). The potential site-specific conjugation was identified by liquid chromatography–mass spectrometry (LC/MS-MS) and the number of linkers per molecule of mAb was calculated using the molecular weight (MW) measured by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The average number obtained in the conjugates in non-reduced conditions was between 8-10 molecules of DOTA per molecule of mAb. The average number obtained in the conjugates in reduced conditions was between 1-2 and 3-4 molecules of DOTA per molecule of mAb in the light chain (LC) and heavy chain (HC) respectively. Potential DOTA modification sites of the chelator were identified in lysine residues. The biological activity of the conjugates was evaluated by flow cytometry (FACS) using CAIX negative (SKRC-18) and CAIX positive (SKRC-52). The DOTA-G250 conjugates were labelled with 177Lu with a radiochemical yield > 95% reaching specific activities of 12 MBq/µg. The stability in vitro of different types of radioconstructs was analyzed in human serum albumin (HSA). The radiostability of 177Lu-DOTA-G250 at high specific activity was increased by addition of sodium ascorbate after the labelling. The immunoreactivity was evaluated in vitro and in vivo. Binding to CAIX positive cells (SK-RC-52) at different specific activities was higher for conjugates with less DOTA content. Protein dose was optimized in mice with subcutaneously growing SK-RC-52 tumors using different amounts of 177Lu- DOTA-G250.

Keywords : mass spectrometry, monoclonal antibody, radiopharmaceuticals, radioimmunotheray, renal cancer
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