RADIOLABELING AND BIODISTRIBUTION STUDY OF ENGINEERED ANTIBODY-LIKE PROTEIN WITH ^{99m}Tc FOR TUMOR THERAPY

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Recently, antibody-like scaffold proteins have received a great deal of interest in diagnosis and therapy applications. Since antibody-like scaffold proteins possess intrinsic features that are often required for tumor imaging and therapy, they have potential to be used as platforms for integrating imaging and therapeutic functions. Intrinsic issues that are associated with therapeutic application of antibody-like scaffold proteins, particularly in cancer treatment, include an efficient and straightforward radiolabeling for understanding in vivo biodistribution and excretion route, and monitoring therapeutic responses. Herein, to investigate the biodistribution and blood clearance, antibody-like scaffold protein, repebody, was successfully radiolabeled with the $[^{99m}Tc(OH_2)_3(CO)_3]^+$ (^{99m}Tc-tricarbonyl) by using a site-specific direct labeling method via hexahistidine-tag, which is a widely used for general purification of proteins with His-affinity chromatography.

Repebody is antibody-like scaffold that consist of highly diverse leucine-rich repeat (LRR) modules, it has been constructed to bind to interleukin-6 for cancer therapy. For ^{99m}Tc labeling to repebody, we first prepared ^{99m}Tc-tricarbonyl presursor. Synthesis of ^{99m}Tc-tricarbonyl precursor achieved more than 90% yields. The result of radio-TLC, the Rf value for ^{99m}Tc-tricarbonyl was 0.0 and 0.3~0.4 using a saline and solvent mixture (methanol 99%, HCl 1%) as the eluent, respectively. And then, the repebody was radiolabeled with ^{99m}Tc-tricarbonyl at 37^{o}C for 1 h. After radiolabeling, for remove unconjugated ^{99m}Tc-tricarbonyl, simple purification step was performed using 10 kDa cut-off filter. Radiochemical purity was determined by radio-TLC. The radiochemical purity of radiolabeled repebody with ^{99m}Tc (^{99m}Tc-repebody) was estimated to be greater than 99% and the specific activity was 925 MBq/mg.

^{99m}Tc-repebody was highly stable up to 24 h for both in vivo SPECT/CT and in vitro histidine challenge study. Biodistribution results showed that most of intraperitoneal doses of ^{99m}Tc-repebody were found in kidneys, and other organs were found with subsequent remaining.

When we examined the whole-body distribution of ^{99m}Tc-repebody via SPECT/CT image analyses, especially after intravenous administration, ^{99m}Tc-repebody was shown to be preferentially presented in the kidney at early time point. Even after 6 h, the radioactivity in the kidney remained high compared to the surrounding tissue. This result indicates the main excretion route of ^{99m}Tc-repebody was through renal clearance. These biodistribution studies of repebody will be useful for development of therapy strategy along with their systemic information.

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