Biodistribution Property of ^{177}Lu Labeled F(ab')$_2$ Fragment of Anti-CD105 for Radioimmunotherapy

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1. Introduction

At the present, most monoclonal antibodies (Abs) on the market or in late-stage development are naked or unconjugated Abs that function by targeting tumor-expressed proteins. An alternative strategy is to use Abs for the targeted delivery of a cytotoxic drug or radionuclide, thereby enhancing the therapeutic efficacy of the Abs. RI labeled Abs have advantages in terms of radiation doses delivered to tumors. For effective radioimmunotherapy(RIT), size is one factor that impacts the circulation time of Ab. Divalent Ab fragments, such as 'minibodies, domain-delated antiodies, or F(ab')$_2$ fragments, combine high accumulation at the tumor site comparable with full Abs, with more rapid clearance from the blood.

In this study, we carried out a radio-immunoconjugation using F(ab')$_2$ fragment of anti-CD105 monoclonal antibody with ^{177}Lu.

2. Methods and Results

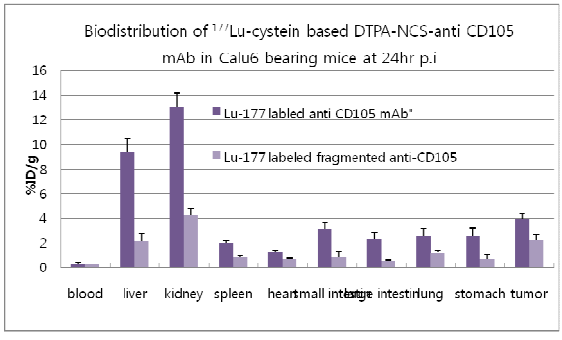
^{177}Lu was produced at the HANARO research reactor (30MW) installed at the Korea Atomic Energy Research Institute (KAERI) by the neutron irradiation of natural ^{176}Lu [^{176}Lu (n, γ) ^{177}Lu]. After the irradiation of a double capsulated $^{176}Lu_2O_3$ target for 5 days at a neutron flux of 1.0 x 10^{14}n/cm^2, it was cooled for 48hrs and dissolved in 3ml of 0.05NHCl solution.

In order to prepare the F(ab')$_2$ fragment of anti-CD105 monoclonal antibody, 50ug of anti-CD105 was added to the spin column containing the immobilized pepsin and incubated for 3 hours at 37℃. The F(ab')$_2$ fragment of anti-CD105 was separated by centrifugation and analyzed in non-reducing SDS-PAGE. Thirty nanograms of radioimmunoconjugate was mixed with 2 ul of the sample buffer (5 × containing 125 mM Tris–HCl (pH 6.8), 4% SDS, 20% glycerol, and 1 ug/ml of bromophenol blue (BPB)) and developed using Hoefer Scientific Instruments electrophoresis apparatus.

The F(ab')$_2$ fragment were conjugated with cysteine based DTPA-NCS at the molar ration of 1:1 at room temperature and for 10 min. For the purification and buffer exchange, Centricon filter system (Millipore) was used in 50mM Na-actetate buffer (pH5.5). The purified immunoconjugates, cysteine based DTPA-NCS-fragmented anti-CD105 was added to 3.7MBq/ml (100uCi/ml) of ^{177}Lu solutions. The reaction mixtures were stirred gently and allowed to stand at room temperature for 10 min. Two to three microliters of ^{177}Lu –DTPA-NCS- F(ab')$_2$ fragment of anti-CD105 mAb were dropped on the ITLC (Instant Thin-Layer Chromatography) silica gel paper (Gelman Science Inc.). ITLC was developed with saline as the mobile phase and analyzed with Cyclone Storage Phosphor System (PerkinElmer). Glassware, materials and solutions for the labeling procedure were sterilized and metal-free. The radioactivity was measure using an ionizing chamber (Capintec 115R, BIODEX Atomlab 200) by setting the calibration value for ^{177}Lu that was corrected and calibrated by the manufacture.

The R_f values of the ^{177}Lu, ^{177}Lu –cysteine based DTPA-NCS, and ^{177}Lu –cysteine based DTPA-NCS-fragmented Ab were 1, 0.8~0.9, and origin, respectively.

For biodistribution study, female BALB/c nude mice (Orient.co), aged 6 to 8 weeks, were injected with 5 x 10^6 Calu6 (human non- small cell lung carcinoma) cells subcutaneously. The mice were used for in vivo biodistribution studies 2 weeks post inoculation of tumor cells, when tumors reached a weight of approximately 0.2g. The 0.185MBq (5uCi) of ^{177}Lu labeled fragment of anti-CD105 was injected intravenously into the tumor bearing mice. For biodistribution studies, the mice were sacrificed 24h (n=5) after injection and the radioactivities in the tumor, kidney, liver, spleen, heart, small and large intestine, lung, stomach and blood were determined using γ-scintillation counter (Perkin Elmer) and expressed as percentage of the injected dose per gram tissue (% ID/g).



3. Conclusions

The F(ab')$_2$ fragment of anti-CD105 was successfully radioimmunoconjugated with ^{177}Lu using cysteine based DTPA-NCS on same procedure of ^{177}Lu labeled full Ab. And we compared the biodistribution properties of ^{177}Lu labeled anti-CD105 and its fragment in human non-small lung cancer cell xenogragts. The ^{177}Lu labeled anti-Cd105 fragment was remained in circulation shorter, significantly increasing tumor accumulation rate and decreasing hematopoietic toxicity. This

radioimmunoconjugate using anti-C105 fragment has a potential for tumor imaging and/or therapy.

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