Radiolabeling of Monoclonal Anti-CD105 with ^{177}Lu for Tumor Diagnosis and Therapy via Angiogenesis Targeting

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1. Introduction

Currently, great interest is focused on angiogenesis and on its potential clinical implications in cancer. An intriguing approach relies on the selective targeting of the surface molecules over-expressed on endothelial cells of tumor-associated blood vessels. CD105 or Endoglin is a cell membrane glycoprotein representing a prime vascular target to implement innovative antibody-based diagnostic and therapeutic strategies. It is a component of the receptor complex of the Transforming Growth Factor (TGF-β), a pleiotropic cytokine involved in cellular proliferation, differentiation and migration.

Radiolabeled monoclonal antibodies have been developed for both the diagnosis and treatment of tumors. One of the ideal radionuclides for imaging and therapy of tumor is ^{177}Lu due to its favorable decay characteristics. ^{177}Lu decays with a half-life of 6.73 d by emission of beta particles with maximum energies of 497 keV (78.6%), 384 keV (9.1%) and 176 keV (12.2%) to stable ^{177}Hf. The emission of gamma photons of 113 keV (6.4%) and 208 keV (11%) with relatively low abundances provides advantages that allow simultaneous scintigraphic studies which helps in proper monitoring the *in vivo* localization of the injected radiopharmaceutical and in performing dosimetric evaluations. In addition, ^{177}Lu can be produced with sufficiently high specific activity using the High-flux Advanced Neutron Application Reactor (HANARO).

In this study, the anti-CD105 mAb was conjugated with the cysteine based isothiocyanatobenzyl-DTPA (DTPA-NCS) as bifunctional chelating agent (BFCA) and labeled with ^{177}Lu. We have previously established the preparation of an amino acid based DTPA by producing 4-Ethylaniline-DTPA-L-Cysteine. Here we describe the successful preparation of ^{177}Lu labeled anti-CD105 mAb using cysteine based DTPA-NCS and investigated its in vitro stability and in vivo biodistribution behavior.

2. Methods and Results

2.1 Preparation of ^{177}Lu labeled anti-CD105 mAb

For our study we chose cysteine based DTPA-NCS as the bifunctional chelating agent, which can be linked to the antibodies via the isothiocynatobenzyl(NCS). The anti-CD105 monoloclonal antibody(BD sciences) was conjugated with DTPA-NCS the molar ratio of 1:1 at room temperature and pH 7.4. Unbound DTPA-NCS was removed by molecular weight filteration system (Millipore). One hundred micromolar of the purified anti-CD105 mAb immunoconjugate was added to the 10MBq/ml of ^{177}Lu solutions resulting to a final pH was 5 to 5.5 and stood for 5 min at room temperature. Radiolabelling yields were determined with Instant Thin-Layer Chromatography (ITLC) scanner (EG&G Berthold linear Analyzer) with silica gel paper (Gelman Science Inc.) using saline as the mobile phase.

The R_f values of the ^{177}Lu, ^{177}Lu -DTPA-NCS, and ^{177}Lu -DTPA-NCS- anti-CD105 mAb were 1, 0.8~0.9, and 0, respectively. The radiolabeling yield was 100% at 5 min. (Fig. 1)

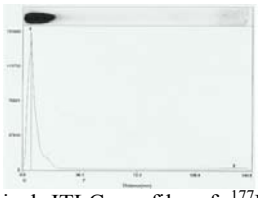

Fig.1. A typical ITLC profile of ^{177}Lu-DTPA-NCS- anti-CD105 antibody determined by ITLC-SG using an autoradiographic system. The *x-axis* distance in cm from origin (left) the *y-axis* shows proportional activity count

The filmless autoradiographies of silica gel or polyacrylamide gel were carried out using Cyclone Storage Phosphor System (PerkinElmer, Wellesley). The radioactivity was measured using an ionizing chamber (Capintec 115R, BIODEX Atomlab 200) by setting the calibration value for ^{177}Lu that was corrected and calibrated by the manufacturer. 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-DTPA-NCS or ^{177}Lu-DTPA-NCS-anti CD105 mAb 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).

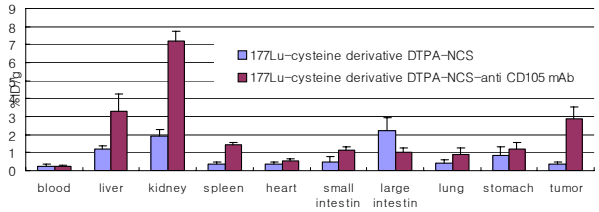

Fig.2. Biodistribution of ^{177}Lu-cysteine based DTPA-NCS-anti CD105 mAb in Calu6 bearing mice at 1 day post injection.

The ^{177}Lu-cysteine derivative DTPA-NCS-anti CD105 had rapid blood clearance, high kidney, liver and tumor uptake, while ^{177}Lu-cysteine derivative DTPA-NCS had no specific accumulation in any organ. The high uptake of the injected ^{177}Lu-cysteine derivative DTPA-NCS-anti-CD105 in the kidneys shows the route of excretion through urinary system. The tumor-to-blood ratio of radioimmunoconjugate was 11.16:1.

3. Conclusions

The anti-CD105 mAb was successfully conjugated with cysteine based DTPA-NCS for 10min at room theperature at pH 7.4 with the molar ratio of 1:1. Then the radioimmunoconjugate was prepared by simple mixing of ^{177}Lu solution with the immunoconjugate at room temperature with high radiolabeling yield($\geq$ 98%).

For the biological evaluations we carried out a biodistribution study using mice bearing Calu 6 lung cancer cell xenografts. The tumor-to-blood ratio was 11.16:1 24h post-injection. In conclusion, the anti-CD105 monoclonal antibody for an angiogenesis targeting was effectively radioconjugated with ^{177}Lu. And the biodistribution study showed a high specificity for accumulating in tumour tissues.

This radioimmunoconjugate is applicable to detect angiogenesis sites in various diseases and to treat tumours.

REFERENCES

[1] Tabata M., Kondo M., Haruta Y., Seon B.K.. Antiangiogenic radioimmunotherapy of human solid tumors in SCID mice using (125)I-labeled anti-endoglin monoclonal antibodies. Int J Cancer 82(5):737-42; 1999

[2] Sun-Ju Choi, Young-Don Hong, So-Young Lee. Therapeutic radionuclides. Nucl Med Mol Imaging. 40(2), 58-65 ; 2006

[3] K.H. Choi, Y.D. Hong, M.S. Pyun and S.J. Choi,, Preparation of an Amino Acid DTPA as as BFCA for Radioimmunotherapy, Bull.Korean Chem. Soc, 27(8), 1194-1198 ; 2006